**UNIVERSIDAD AUTÓNOMA DE MADRID**

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# PÉPTIDOS IMPLICADOS EN LA REGULACIÓN DE LA HOMEOSTASIS DE LA ENERGÍA E INFLAMACIÓN CRÓNICA DE BAJO GRADO EN EDAD PEDIÁTRICA

OLAYA DE DIOS HUERTA

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Departamento de Pediatría

Facultad de Medicina

Universidad Autónoma de Madrid

# Péptidos implicados en la regulación de la homeostasis de la energía e inflamación crónica de bajo grado en edad pediátrica

Memoria de investigación presentada por

**Olaya de Dios Huerta**

Licenciada en Biología

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Trabajo dirigido por:

**Dra. Carmen Garcés Segura**

y

**Prof. Leandro Soriano Guillén**

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A todos aquellos que siempre

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# RESUMEN

Dentro del origen multifactorial de la obesidad, la regulación de la homeostasis de la energía constituye un factor clave en su desarrollo. Así, el hipotálamo desempeña un papel fundamental en el control del equilibrio energético, respondiendo tanto a señales centrales como periféricas que informan sobre el estado energético, modulando la ingesta de alimentos, el gasto energético y el metabolismo de los hidratos de carbonos y de los lípidos. La obesidad se caracteriza por una acumulación excesiva de tejido adiposo que provoca una alteración de sus funciones endocrinas y un estado de inflamación crónica de bajo grado que desencadena el desarrollo de comorbilidades como el síndrome metabólico.

En nuestro estudio, en edad pediátrica, hemos profundizado el papel de leptina e insulina como señales de adiposidad implicadas en la regulación de la ingesta dietética y de nesfatina-1 y adropina como "nuevas señales" que podrían estar implicadas en la homeostasis de la energía. Además, hemos ahondado en el estudio de la proteína C-reactiva (PCR) como marcador de inflamación crónica, investigando factores reguladores de sus niveles plasmáticos, y su probable implicación en la etiopatogenia de alteraciones metabólicas.

Nuestros resultados han demostrado una acción anorexigénica de leptina dependiente de los valores sanguíneos de insulina en niñas. También hemos evidenciado una relación de la obesidad tanto con los niveles de nesfatina-1 como con los de adropina, presentando ambos péptidos una diferente regulación dependiente del sexo. La asociación observada de leptina con nesfatina-1 y adropina sugiere la posible regulación de la secreción de estos péptidos a nivel central por la leptina. Con referencia a la PCR como marcador de inflamación crónica y de alteraciones metabólicas, hemos confirmado que el tejido adiposo es una fuente extrahepática de PCR, cuya expresión estaría asociada potencialmente con la gravedad de la inflamación. Al analizar la validez de PCR como marcador de síndrome metabólico, hemos constatado su utilidad como biomarcador en niñas adolescentes, pero no en niños, en los que su asociación parece estar influenciada por leptina. Siguiendo esta línea, en esta misma población, hemos observado que la leptina también participaría como mediador de la acción inflamatoria de la testosterona en los niños adolescentes. Por último, hemos demostrado que, a diferencia de otros antioxidantes liposolubles, los niveles de retinol plasmático se asocian negativamente de manera significativa con las concentraciones de PCR.

En conclusión, nuestros datos corroboran la existencia de un importante dimorfismo sexual en la fisiopatología de la obesidad que ha de tenerse en cuenta ya en la edad pediátrica.

# SUMMARY

Obesity has a multifactorial origin, with the regulation of energy homeostasis being a key factor in its development. Thus, the hypothalamus plays a fundamental role in the control of the energy balance, responding to central and peripheral signals that inform about the energy status, and modulating the food intake, the energy expenditure and the carbohydrates and lipids metabolism. Obesity is characterized by an excessive accumulation of adipose tissue that provokes an alteration of its endocrine functions and a low-grade chronic inflammation state, triggering the development of comorbidities such as the metabolic syndrome.

In our study, carried out in pediatric age, we have investigated in deep the role of leptin and insulin as adiposity signals involved in the regulation of the food intake and the role of nesfatin-1 and adropin as "new signals" that could be involved in energy homeostasis. Additionally, we have deepened in the study of C-reactive protein (CRP) as a marker of chronic inflammation, investigating possible regulatory factors of its plasma levels and its probable involvement in the etiopathogenesis of different metabolic alterations.

Our results have demonstrated an anorexigenic leptin action depending on the blood insulin levels in girls. We have also evidenced a relationship of obesity with nesfatin-1 and adropin levels, showing both peptides a different regulation depending on sex. The association found between leptin with both nesfatin-1 and adropin suggests the possible regulation of the secretion of these peptides at central level by leptin. Regarding CRP as a marker of chronic inflammation and metabolic alterations, we have confirmed that the adipose tissue is an extrahepatic source of CRP, and its expression would be potentially related to the severity of inflammation. When analyzing the validity of CRP as a marker of metabolic syndrome, we have confirmed its utility in adolescent girls, but not in boys in whom its association with metabolic syndrome seems to be influenced by leptin. Moreover, in this population, we have observed that leptin would also participate as a mediator of the inflammatory action of testosterone in adolescent boys. Finally, we have demonstrated that, unlike other soluble fat antioxidants, plasma retinol levels are significantly negatively associated with CRP concentrations.

In conclusion, our data bear out the existence of an important sexual dimorphism in the physiopathology of obesity which should be taken into account already at the pediatric age.

# ÍNDICE

# ACRÓNIMOS Y ABREVIATURAS

Actividad física, sedentarismo y obesidad en la juventud española (**PASOS** - *Physical Activity, Sedentarism and Obesity in Spanish Youth* )

Adiponectina (**ADIPOQ**)

Cuatro Provincias (**4P**)

Factor de necrosis tumoral (**TNF** - *Tumour Necrosis Factor*)

Federación Internacional de Diabetes (**IDF** - *International Diabetes Federation*)

Federación Mundial de Obesidad (**FMO**)

Globulina transportadora de hormonas sexuales (**SHBG** - *Sex Hormone-Binding Globulin*)

Grupo de trabajo internacional sobre obesidad (**IOTF** - *International Obesity Task Force*)

Homeostasis energética asociada (**Enho** - *Energy Homeostasis Associated*)

Índice de estradiol libre (**FEI** - *Free Estradiol Index*)

Índice de masa corporal (**IMC**)

Iniciativa de vigilancia de la obesidad infantil (**COSI** - *Childhood Obesity Surveillance Initiative*)

Interleucina (**IL**)

Leptina (**LEP**)

Neuropéptido Y (**NPY**)

Núcleo arcuato (**ARC**)

Nucleobindina-2 (**NUCB2**)

Organización Mundial de la Salud (**OMS**)

Péptido relacionado con agutí (**AgRP** - *Agouti-related protein*)

Pro-opiomelanocortina (**POMC**)

Proteína C-reactiva (**PCR**)

Proteínas desacoplantes de tipo 1 (**UCP1** - *Uncoupling Protein* 1)

Receptor de insulina (**InsR** - *Insulin Receptor*)

Receptor de leptina (**LepR** - *Leptin Receptor*)

Síndrome de ovario poliquístico (**PCOS** - *Polycystic Ovary Syndrome* )

Síndrome metabólico (**SMet**)

Sustrato receptor de la insulina-2 (**IRS2** - *Insulin Receptor Substrate-2*)

Tejido adiposo (**TA**)

Tejido adiposo blanco (**TAB**)

Tejido adiposo marrón (**TAM**)

Tejido adiposo subcutáneo (**TAs**)

Tejido adiposo visceral (**TAv**)

Transcripción regulada por cocaína-anfetamina (**CART** - *Cocaine- and Amphetamine-Regulated Transcript*)

Triacilglicerol (**TAG**)

# 1. OBESIDAD

Según la Organización Mundial de la Salud (OMS), la obesidad se define como una acumulación anormal o excesiva de grasa que favorece la aparición de otras enfermedades[1], y la Federación Mundial de Obesidad (FMO) considera a la obesidad como una auténtica enfermedad crónica[2].

## CLASIFICACIÓN DEL EXCESO DE PESO: SOBREPESO Y OBESIDAD

En la actualidad, los dos métodos indirectos más ampliamente utilizados para estimar el exceso de peso a nivel poblacional son el índice de masa corporal (IMC) y el perímetro de la cintura.

El IMC (IMC= $Kg/m^2$) es el método indirecto más aceptado y empleado para la estimación de la obesidad tanto en población adulta como pediátrica. En el adulto, los puntos de corte del IMC fueron propuestos en un informe de la OMS[3], definiéndose el sobrepeso como un IMC entre 25 y 29,9 y la obesidad como un IMC mayor o igual a 30. En población infantil, una de las clasificaciones más empleadas son los puntos de corte internacionales de la IOTF (*International Obesity Task Force)* de *Cole y col*.[4]. Esta clasificación se basa en datos internacionales combinados para IMC que mediante el método de mínimos cuadrados promedio, fija puntos de corte del IMC específicos por edad y sexo, desde los 2 a los 18 años[4]. Las tablas se actualizaron en el año 2012 introduciéndose algunos cambios que permitieron comparar estas tablas con otro tipo de clasificaciones que emplean percentiles o Z-score (desviaciones estándar respecto a la media), sin afectar a las estimaciones previas de prevalencia de sobrepeso y obesidad[5]. Actualmente, la FMO recomienda el uso los puntos de corte internaciones de la IOTF[6].

El perímetro de la cintura es otro parámetro antropométrico indirecto de medición de la obesidad central ampliamente empleado. La circunferencia de la cintura se considera un indicador razonable de grasa abdominal o visceral empleado como medida para la identificación de pacientes pediátricos con alteraciones cardiometabólicas y como herramienta diagnóstica para detectar poblaciones en riesgo[7,8]. Así, la Federación Internacional de Diabetes (IDF) considera el perímetro de la cintura un factor indispensable para la definición de síndrome metabólico (SMet), estableciendo el punto de corte en edad adulta en 94 centímetros para los hombres y 80 para las mujeres. En la edad pediátrica se establece el punto de corte en valores por encima del percentil 90 para la edad y el sexo[9]. El principal problema de la utilización del perímetro de cintura en la edad pediátrica lo constituye la escasez de datos con percentiles teniendo en cuenta la edad y el sexo para la población de referencia. En España, las tablas de referencia más empleadas son las de *Fernández y col*.[10].

## 1.1. LA OBESIDAD COMO PROBLEMA DE SALUD PÚBLICA

El entorno obesogénico de las últimas décadas, basado en una actividad física limitada y un aumento de la ingesta calórica, debido a la cantidad (disponibilidad teóricamente ilimitada) como por la calidad (alta en grasas y azúcar) de la dieta, ha contribuido al incremento exponencial de la prevalencia de sobrepeso y obesidad constituyendo un enorme problema de salud pública[11].

### 1.1.1. EPIDEMIOLOGÍA DE LA OBESIDAD

En 2016, más de 1.900 millones de adultos presentaban exceso de peso. De éstos, más de 650 millones eran obesos[12]. Esto significa que aproximadamente el 39% de la población mundial adulta presentaba sobrepeso y el 13% obesidad[12].

La obesidad y el sobrepeso son factores de riesgo importantes en enfermedades no transmisibles como diabetes tipo II, trastornos musculoesqueléticos, algunos cánceres (especialmente el de endometrio, mama, ovario, próstata, hígado, vesícula biliar, riñón y colon) y enfermedades cardiovasculares[12], siendo esta última la principal causa de muerte, tanto en hombres como en mujeres, en el año 2019[13].

Si se tiene en cuenta que la obesidad infantil es un importante predictor de obesidad en el adulto[14], la obesidad en la infancia y en la adolescencia se ha convertido en uno de los problemas de salud pública más relevantes del siglo XXI[15]. De esta forma, la prevalencia de exceso de peso ha aumentado a un ritmo alarmante, del 4% en 1975 a más del 18% en 2016 lo que significa que más de 340 millones de niños y adolescentes (de 5 a 19 años) presentan sobrepeso u obesidad[15]. Así, la OMS considera la obesidad infantil una epidemia global, siendo uno de los factores de riesgo más preocupantes para la salud pública en las próximas generaciones[16].

En el marco europeo, los últimos datos que muestra la OMS provienen de resultados de la Iniciativa de Vigilancia de la Obesidad Infantil (COSI, *Childhood Obesity Surveillance Initiative*)[17]. Este informe revela que en Europa los países mediterráneos son los que presentan una mayor prevalencia de sobrepeso y obesidad infantil, tanto en niños como en niñas, en edades comprendidas entre los 5 y los 10 años[17]. En este estudio, España se encuentra entre los cinco países con mayor prevalencia de exceso de peso, ocupando un preocupante segundo lugar en las niñas y un cuarto lugar en los niños (Figura 1)[17].

En España, uno de los últimos estudios epidemiológicos de obesidad infanto-juvenil (8 a 16 años) es el estudio PASOS 2019 (*Physical Activity, Sedentarism and Obesity in Spanish Youth*)[18]. En su informe describe, tomando en consideración el IMC, una prevalencia de exceso de peso

de un 34,9%, distribuida de la siguiente forma: un 20,7% de niños/as y adolescentes presenta sobrepeso y un 14,2% obesidad (Figura 2A)[18]. No obstante, tomando como referencia la circunferencia de la cintura, los datos mostraron una prevalencia de obesidad del 24,6%, lo que supone un aumento de un 10,3% respecto a los resultados presentados según el IMC (Figura 2B)[18].

Este mismo informe muestra un aumento de la prevalencia de obesidad infantil en las dos últimas décadas de un 1,6% según IMC y un 8,3% teniendo en cuenta la cintura. En suma, España sufre una auténtica epidemia de obesidad infantil que está afectando de forma relevante al desarrollo de los niños y adolescentes de nuestro entorno[18].

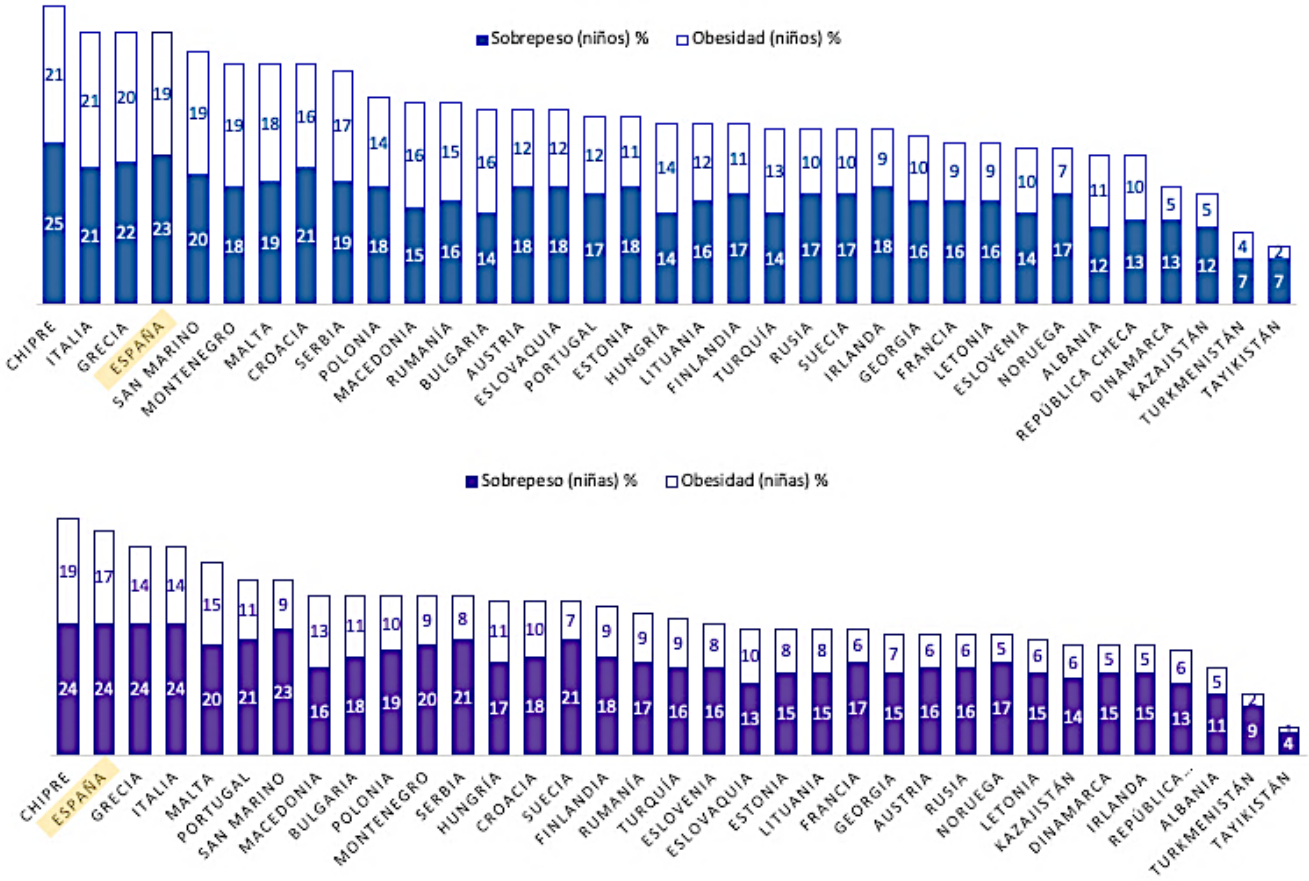


**Figura 1.** Prevalencia de exceso de peso en niños y niñas en Europa. Datos extraídos del estudio COSI[17].

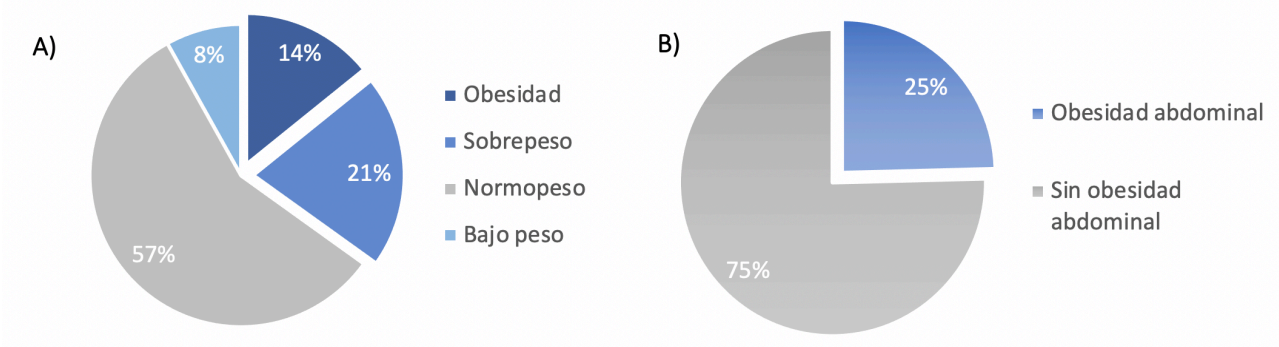


**Figura 2.** Prevalencia de sobrepeso y obesidad en España según el índice de masa corporal (IMC) (A) y el perímetro de la cintura (B). Datos extraídos del informe final del estudio PASOS[18].

### 1.1.2. Obesidad infantil y riesgo cardiovascular en el adulto

El sobrepeso y la obesidad infantil pueden traer consigo importantes consecuencias a corto y largo plazo[19]. En esta línea, ya desde edades tempranas se ha constatado la presencia de factores de riesgo cardiovascular como dislipemia, hipertesión arterial y alteración del metabolismo hidrocarbonado, entre otros. Estas comorbilidades muestran una asociación directa con el aumento del IMC y de la circunferencia de la cintura[19]. Además, ya en la infancia, se observa que la obesidad puede desencadenar y mantener un estado de inflamación crónica de bajo grado que parece estar relacionado con la aparición de resistencia a la insulina y SMet[20].

Se sabe que la obesidad infantil condiciona la presencia de obesidad a lo largo de la vida y, en consecuencia, favorece la aparición de distintas comorbilidades. Nuestro grupo mostró que el 75% de los niños/as de 6-8 años mantenían la misma categoría de peso a los 13-16 años[21]. Otro estudio ha demostrado que el 75% de los niños/as que tenían un IMC por encima del percentil 70 a los 5 años presentaban un exceso de peso en la adolescencia[22]. Esta tendencia se prolonga en el tiempo de tal forma que entorno al 80% de los adolescentes obesos seguirán siendo obesos en la transición a la etapa adulta y alrededor del 70% serán obesos después de los 30 años[14]. Además, la evidencia científica existente señala que la presencia prolongada de sobrepeso y obesidad incrementa la morbimortalidad prematura en la edad adulta[23].

En este contexto, nuestro grupo puso en marcha el estudio Cuatro Provincias (4P), en el que se comparan factores de riesgo cardiovascular en población pediátrica de cuatro provincias españolas con distintas tasas de mortalidad cardiovascular en el adulto, demostrando que las provincias con mayor prevalencia de obesidad infantil se relacionaban con las provincias de mayor mortalidad en la edad adulta[24].

Por tanto, la edad pediátrica constituye un momento clave para prevenir la aparición de sobrepeso y la obesidad y, por ende, evitar el desarrollo de comorbilidades.

## 1.2. FISIOPATOLOGÍA DE LA OBESIDAD

La obesidad tiene un origen multifactorial, de modo que factores ambientales en estrecha relación con factores genéticos de riesgo subyacentes contribuyen a un desequilibrio entre ingesta y gasto calórico. El hipotálamo es una región clave del cerebro en la regulación del equilibrio energético, ya que controla tanto la ingesta de alimentos a merced de la regulación de apetito/saciedad, como el almacenamiento y el gasto de energía mediante la integración de señales tanto centrales como periféricas[25].

Este desequilibrio en el balance energético causa una acumulación excesiva de grasa y la posible pérdida de la funcionalidad del tejido adiposo que implica un desajuste en sus funciones metabólicas y homeostáticas. Todo ello contribuye a la generación de un estado de inflamación crónica de bajo grado y al desarrollo de las patologías asociadas a la obesidad[26]**.**

En los siguientes apartados ahondaremos en la fisiopatología de la obesidad, centrándonos en la influencia de ciertos péptidos sobre la regulación de la ingesta y la homeostasis de la energía y en la pérdida de la funcionalidad del propio tejido adiposo.

## 2. REGULACIÓN DE LA HOMEOSTASIS DE LA ENERGÍA

El hipotálamo desempeña un papel clave en el control de equilibrio energético, respondiendo a señales centrales y periféricas que informan acerca del estado energético, modulando la ingesta de alimentos, el gasto energético y el metabolismo de la glucosa y de los lípidos en distintos órganos, como el hígado, el músculo esquelético o el tejido adiposo, a través del sistema nervioso autónomo[27].

La leptina y la insulina son dos señales periféricas "clásicas" que modulan el metabolismo energético[28]. Ambas moléculas son consideradas señales de adiposidad ya que sus niveles son proporcionales a la cantidad de grasa corporal total e informan al sistema nervioso central sobre el estado de energía almacenada a través de sus respectivos receptores expresados en áreas clave del hipotálamo[29,30].

Además de estas señales "clásicas", en los últimos años han aparecido "nuevas" señales tanto centrales como periféricas que parecen tener un rol importante en la homeostasis energética. Entre ellas, dos péptidos han adquirido una trascendencia especial en la regulación de la homeostasis energética: nesfatina-1 y adropina.

La nesfatina-1 se detectó por primera vez en los núcleos cerebrales reguladores de la ingesta de alimentos, constituyendo una potente señal central anorexigénica[31]. Posteriormente, esta molécula también se ha detectado en tejidos periféricos (mucosa gástrica, tejido adiposo y las células beta pancreáticas, entre otros) modulando distintas acciones entre las que destacan funciones gastrointestinales, del metabolismo de la glucosa y los lípidos, la termogénesis, así como cardiovasculares y reproductivas[32].

La adropina se sintetiza principalmente en el hígado y el cerebro[33], aunque también se localiza en otros tejidos periféricos como el corazón y el tracto gastrointestinal[34]. Es una proteína codificada por el gen *Enho (*Energy Homeostasis Associated) cuya expresión se ve reducida por la obesidad y regulada por la composición de la dieta[33]. Su principal función se ha relacionado con la regulación del metabolismo de la glucosa y de los lípidos, pudiendo ejercer efectos metabólicos beneficiosos en modelos animales de obesidad además de mejorar la función del sistema cardiovascular[34].

En los siguientes puntos se profundizará en la leptina y la insulina como señales periféricas "clásicas" marcadoras de adiposidad y, en la nesfatina-1 y la adropina, como "nuevas" señales reguladoras de la homeostasis energética.

## 2.1. LEPTINA E INSULINA

La leptina y la insulina se consideran dos señales periféricas clave en la homeostasis energética que actúan en el hipotálamo y otras áreas del cerebro[35]. La insulina fue la primera hormona que se identificó como señal de adiposidad[28]. Se secreta por las células β pancreáticas en respuesta a los niveles de glucosa y sus concentraciones en plasma aumentan en proporción a la cantidad de grasa almacenada[28]. La leptina fue descubierta, en el año 1994, por clonación posicional del locus de obesidad (ob). Esta hormona se produce principalmente en el tejido adiposo blanco (TAB), siendo la cantidad de masa grasa el principal factor determinante de sus niveles plasmáticos, por lo que se considera un buen marcador de adiposidad[28].

Estas dos moléculas tienen la capacidad de atravesar la barrera hematoencefálica y actuar sobre sus respectivos receptores en las áreas de control de la ingesta, específicamente sobre el núcleo arcuato (ARC)[36]. En esta región podemos encontrar neuronas de acción anorexigénica que expresan pro-opiomelanocortina (POMC) y transcripto regulado por cocaína-anfetamina (CART); y las neuronas de acción orexigénica que producen neuropéptido Y (NPY) y péptido relacionado con agutí (AgRP). En las neuronas POMC/CART, la acción de la insulina y la leptina se considera estimulante para promover la saciedad, mientras que en las neuronas NPY/AgRP el efecto de la señalización es principalmente inhibitorio reduciendo el apetito y aumentando el gasto energético[37] (Figura 3).

La acción anorexigénica de cada una de estas dos señales por separado ha sido ampliamente demostrada. Un estudio en ratones mostró que la administración intracerebroventricular tanto de leptina como de insulina redujo la ingesta de alimentos y el peso corporal[38]. Los efectos anorexigénicos de estas hormonas se encuentran mediados por los receptores de leptina e insulina expresados en el sistema nervioso central[39,40]. Así, la deleción neuronal del receptor de insulina (InsR) y del sustrato receptor de la insulina-2 (IRS2) produce un aumento de la ingesta de alimentos y una mayor susceptibilidad a la obesidad inducida por la dieta [40–42]. Mientras que la deficiencia genética de leptina o del receptor de leptina (LepR) se ha asociado con hiperfagia, hipoactividad y obesidad[43]. En humanos, la deficiencia congénita de leptina y/o su receptor se ha relacionado con obesidad mórbida de inicio temprano[44] y con hiperfagia[45].

El estudio de *Air y col*.[46] fue el primero en analizar simultáneamente el papel de ambas señales en la regulación de la ingesta alimentaria y el peso corporal, demostrando que, en ratas, la administración concomitante de leptina e insulina a nivel intracerebroventricular disminuyó la ingesta de alimentos y el peso de forma aditiva. Posteriormente, *Kleinridders A y col*.[47] demostraron que, en algunas áreas hipotalámicas, la insulina y la leptina activan cascadas

enzimáticas superpuestas, y que la acción anorexigénica de cada una podría inhibir o estimular a la otra. Estas evidencias sugieren la presencia de un "diálogo cruzado" entre la leptina y la insulina a nivel central[37,48,49].

Además de la posible interacción sobre el sistema nervioso central, son varios los estudios que muestran una interacción directa insulina-leptina a nivel periférico. Desde el estudio de *Barr y col*.[50] en el que demostraron que la insulina estimulaba tanto la secreción como la producción de leptina en el TAB, son numerosos los estudio tanto *in vitro* como *in vivo* que han respaldado estos resultados. Uno de los últimos estudios, muestra *in vivo,* una acción directa de la insulina estimulando la secreción de leptina y la expresión de ARNm de *leptina*[51]. Por otra parte, se ha demostrado la presencia de receptores de leptina en las células β pancreáticas[52], a través de los cuales la leptina inhibiría la expresión de *insulina*[53]. La leptina también podría inhibir la secreción de insulina, tanto basal como la estimulada por glucosa, a través de los canales de potasio dependientes de ATP[53].

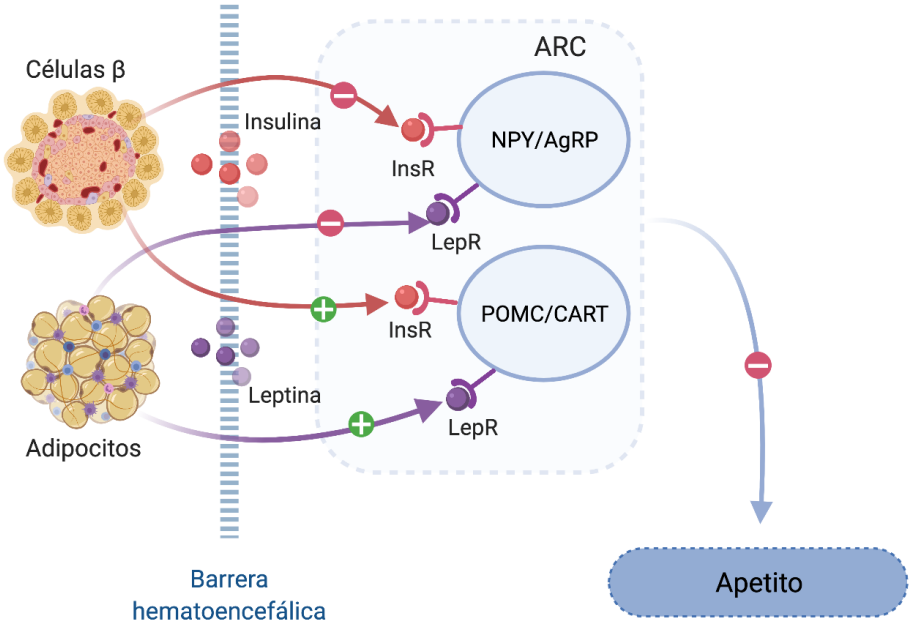


**Figura 3.** Resumen de la ruta de señalización en el hipotálamo de la leptina y la insulina. La leptina y la insulina secretadas por el tejido adiposo y el páncreas, respectivamente, informan al hipotálamo sobre el estado energético del organismo. Cuando LepR e InsR son activados por estas hormonas, promueven cambios en la expresión de neuropéptidos hipotalámicos, que provocan alteraciones en las funciones periféricas para restaurar el equilibrio energético y el metabolismo de la glucosa. InsR, receptor de insulina; LepR, receptor de leptina ; ARC, núcleo arcuato; AgRP, proteína relacionada con agutí; NPY, neuropéptido Y; POMC, proopiomelanocortina; CART, transcriptor relacionado con cocaína-anfetamina . Figura creada con: BioRender.com.

Por tanto, el efecto que puede tener leptina sobre la insulina y, viceversa, podría contribuir a explicar, al menos en parte, los resultados contradictorios observados en distintos estudios poblacionales sobre la asociación de los niveles de leptina con la ingesta energética[54–63].

## 2.2. NESFATINA-1

En el año 2006 *Oh-I y col.*[31] descubrieron una nueva hormona anorexigénica a la que denominaron nesfatina-1. La nesfatina-1 es un péptido de 82 aminoácidos originado a partir del procesamiento postraduccional de la proteína de 396 aminoácidos, nucleobindina-2 (NUCB2), cuyo procesamiento también produce nesfatina-2 y nesfatina-3[31], dos péptidos con funciones hasta ahora desconocidas[32].

Nesfatina-1, se encontró por primera vez en el núcleo hipotalámico regulador de la ingesta de alimentos y, posteriormente, se detectó en otros núcleos cerebrales tanto de rata[31] como de ratón[64]. En un principio se localizó principalmente en el soma y las dendritas primarias de las neuronas, detectando menos inmunorreactividad en las fibras nerviosas[65], lo que apuntaba a un modo de acción autocrino y/o paracrino en lugar de endocrino. Así, este neuropéptido parece jugar un papel importante en la regulación del apetito, además, de estar implicado en la regulación de la reproducción, el sueño, la cognición y las respuestas relacionadas con la ansiedad o el estrés[66,67].

Son varios los estudios en modelos animales que han relacionado la nesfatina-1 con la ingesta de alimentos y la saciedad[64]. En estos estudios, tanto en ratas[68–70] como ratones[65,71], se demostró que la inyección intracerebroventricular de NUCB2 reducía la ingesta de alimentos de una manera dependiente de la dosis, dando como resultado una reducción del peso corporal[72]; mientras que la inyección de un anticuerpo neutralizador de la nesfatina-1 provocaba la estimulación del apetito[31].

Por otra parte, también se ha demostrado que la nesfatina-1 atraviesa la barrera hematoencefálica en ambas direcciones mediante un mecanismo no saturable[73], expresándose ampliamente en tejidos periféricos[74]. El estómago se ha postulado como la principal fuente de nesfatina-1 circulante debido a su alta expresión génica tanto en estudios animales como en humanos[74]. Además, se ha constatado la co-localización de ghrelina y nesfatina-1 en células gástricas humanas[75], produciéndose una liberación diferencial de estos dos péptidos para estimular o inhibir la ingesta de alimentos[76]. En el tejido adiposo (TA) también se detectó de manera consistente la expresión génica y proteica de nesfatina-1 en TA murino y humano[77], presentando una mayor expresión en el TA subcutáneo (TAs) que en el TA visceral (TAv)[77]. Más

recientemente se ha sugerido un aumento de la expresión de *nesfatina-1* en el TA marrón (TAM) asociado a un incremento del gasto energético[78]. Todas estas evidencias respaldan la relevancia de la regulación periférica de nesfatina-1.

En modelos animales, se ha visto que la administración periférica de nesfatina-1 inhibe la ingesta de alimentos en ratones de manera independiente de leptina[79]. De manera similar, la infusión subcutánea crónica de nesfatina-1 redujo la ingesta de alimentos y moduló la homeostasis de la energía corporal en ratas[80]. Si bien los estudios en modelos animales son consistentes, los estudios en humanos muestran resultados contradictorios. De esta forma, varios trabajos han mostrado correlaciones negativas entre los niveles de nesfatina-1 y el IMC tanto en adultos[81,82] como en niños[83,84]; mientras que otros no han encontrado ninguna asociación[85–87] o, por el contrario, han descrito una relación positiva tanto en adultos[77,88] como en edad infantil[89,90]. Esta discrepancia podría deberse, además de a otros factores de confusión aún por definir, a una influencia del sexo ya que se han objetivado valores más elevados de nesfatina-1 circulante en mujeres en comparación con los hombres[86]. A su vez, este dimorfismo sexual observado en los valores de nesfatina-1 podría ser explicado por una posible influencia de las hormonas esteroideas sexuales y/o por el distinto porcentaje de masa grasa entre hombres y mujeres[74].

Por otra parte, algunos estudios han investigado una posible modulación de los niveles de nesfatina-1 por parte de la ingesta de alimentos[68,76]. Sin embargo, las evidencias sobre el tipo de componentes dietéticos (carbohidratos, proteínas y grasas ) que desencadenarían la expresión de nesfatina-1 son escasos. En modelos animales, se ha visto que ciertos componentes de los alimentos podrían regular los niveles de nesfatina-1[91–93]; sin embargo, en los seres humanos la regulación de la nesfatina-1 endógena a través de la dieta sigue sin estar suficientemente aclarada[90].

En suma, la nesfatina-1 se considera un péptido con una importante relevancia en la homeostasis de la energía a través de efectos centrales y periféricos. Los resultados contradictorios observados en humanos apuntan a la necesidad de seguir investigando la influencia de factores reguladores de la síntesis y liberación de nesfatina-1.

### 2.3. ADROPINA

En 2008 *Kumar y col*.[33] identificaron un nuevo péptido al que denominaron adropina. Los autores de este trabajo, mediante el análisis de microarrays de expresión génica en hígado de ratones obesos, hallaron un nuevo producto de transcripción hepática del gen *Enho* que se

encontraba regulado negativamente en la obesidad. La adropina es un péptido compuesto por 43 aminoácidos fruto de una escisión proteolítica de un precursor de 76 aminoácidos. Es importante destacar que la secuencia de aminoácidos de la adropina está altamente conservada entre las especies, siendo idéntica en ratas, ratones, humanos y cerdos[33]. Por otra parte, conviene señalar que la vida media plasmática de la adropina se encuentra aún en fase de investigación[34].

La adropina se localiza principalmente en el cerebro y el hígado[33,94,95], aunque en los últimos años también se ha detectado en otros tejidos periféricos como el corazón, pulmón, médula renal y músculos[34].

En cuanto a su mecanismo de acción, en un principio se propuso que sus efectos biológicos podrían estar mediados por la interacción con el receptor acoplado a proteína G, el receptor GPR19[96,97]; sin embargo, otro estudio indicó que la adropina es una proteína de membrana plasmática que modula la actividad física y la coordinación motora a través de la señalización de la vía NB-3/Notch[95]. De este modo, la adropina podría actuar tanto como factor de secreción como proteína de membrana.

Sobre su funcionalidad, varios estudios en roedores se centraron en el papel de adropina en el metabolismo de la glucosa y de los lípidos. *Kumar y col*. demostraron que el tratamiento con adropina mejoraba la sensibilidad a la insulina y el perfil lipídico de roedores[33,98]. *Akcılar R y col*. también mostraron que la adropina aumentaba la sensibilidad a la insulina, reducía la hiperglucemia, además de disminuir la expresión de citoquinas proinflamatorias (TNF-α e IL-6), en modelos animales de diabetes tipo 2[99]. *Gao y col.* propusieron al músculo esquelético como un órgano clave en la mediación de los efectos de la adropina en todo el organismo[100,101], siendo un mecanismo dependiente de la síntesis de glucosa hepática[102]. Por último, se ha demostrado que la adropina puede interactuar con el TAB , reduciendo la expresión de genes lipogénicos[33] y la acumulación de lípidos[101].

Mientras que los estudios en animales son consistentes, los estudios en humanos son escasos y con resultados dispares. Uno de los primeros estudios fue el realizado por *Butler y col.* que investigaron la asociación entre los niveles plasmáticos de adropina con la presencia de obesidad en adultos, encontrando que la adropina se relacionaba positivamente con la mejora del perfil lipídico y negativamente con el IMC[103]. Posteriormente, varios estudios en adultos confirmaron esta asociación inversa entre los valores sanguíneos de adropina y el IMC[104–107], mostrando también niveles más bajos de adropina en pacientes con SMet[105]. Sin embargo, *Ghoshal y col*.[108] demostraron en hombres jóvenes delgados una asociación positiva entre los

niveles de adropina e IMC, aunque en este mismo estudio también vieron que el aumento de los niveles circulantes de adropina era un factor de riesgo de obesidad en las etapas media y tardía de la vida. Por lo que podría haber factores de confusión, como la edad o el género, que afectarían a los valores sanguíneos de adropina. Por otra parte, en adultos se han descrito correlaciones negativas entre adropina y edad[94,109] y niveles plasmáticos más altos en las mujeres que en los hombres[103]. Finalmente, la relación observada entre adropina y resistencia a la insulina en estudios animales, no se ha documentado claramente en humanos[110–112].

Hasta la fecha, en la edad pediátrica, son escasos los estudios que hayan investigado la relación entre adropina, obesidad y alteraciones metabólicas[104,113–116] (Tabla 1), siendo necesario un mayor estudio de los factores que podrían influir en su síntesis.

**Tabla 1.** Resumen de los principales estudios publicados en edad pediátrica que han analizado la posible asociación de adropina y la presencia de obesidad .

| Autores (Ref) | Sujetos (n) | Tipo de estudio | Edad | Asociación con obesidad |
|---|---|---|---|---|
| *Sayın y col.*[104] | 100♂/♀ | 34 OB/30 EHGNA/36 NP | 12.9 ± 2.1 | Niveles más altos de adropina en el grupo NP. |
| *Kocaoglu y col.*[113] | 70♂/♀ | 42 OB/28 SMet/26 NP | 13.8±1.9 | Resultados no significativos |
| Altinciky col.[114] | 55♂/♀ | 40 OB/15 NP | 7.5–16.4 | Niveles más altos de adropina en el grupo NP. |
| *Zhang y col.*[115] | 60♂/♀ | 45 OB/20 NP | 16–19 | Niveles más altos de adropina en el grupo NP. |
| *Chang y col.*[116] | 269♂/223♀ | Estudio transversal | 12–15 | Los niveles más altos de adropina se asocian a un ratio cintura-cadera más baja y un porcentaje de grasa corporal más bajo. |

♂: niños; ♀: niñas; OB: obesos; NP: normopeso; EHGNA: enfermedad del hígado graso no alcohólico; SMet: síndrome metabólico

## 3. OBESIDAD Y DISFUNCIÓN DEL TEJIDO ADIPOSO

La obesidad implica una pérdida de funcionalidad del tejido adiposo generando una alteración en sus funciones endocrinas y un estado de inflamación crónica de bajo grado que provoca el desarrollo de patologías asociadas a la obesidad[117,118].

Hasta el descubrimiento de la leptina, se desconocía la función endocrina e inmunitaria del tejido adiposo (TA) y se consideraba un depósito inactivo de energía acumulada en forma de triglicéridos[119]. A día de hoy, se sabe que las sustancias secretadas por este tejido participan en el control del metabolismo energético, lipídico y de los carbohidratos y pueden modular la actividad del sistema inmunológico[120].

### 3.1. CARACTERÍSTICAS GENERALES DEL TEJIDO ADIPOSO

El TA se considera un tejido conectivo formado por diferentes tipos de células: adipocitos, que en condiciones fisiológicamente normales representan un tercio del tejido, fibroblastos, macrófagos, células estromales, monocitos y preadipocitos[119]. Según la morfología, disposición y función, el TA puede clasificarse en tejido adiposo blanco (TAB), marrón (TAM), beige y rosa[121]. Las principales diferencias entre el TAB, TAM y beige se encuentran resumidas en la Figura 4. El cuarto tipo de tejido, formado por adipocitos rosas que se encuentran en glándulas mamarias lactantes de los mamíferos hembras[122], no estaría implicado en la obesidad.

Las dos formas principales de TA son el TAB y el TAM. El TAB es el depósito de grasa más abundante y actúa como la principal reserva del excedente de energía en el cuerpo, almacenando el exceso de nutrientes en forma de triacilglicerol, además de tener una importante función endocrina[123]. La morfología de los adipocitos blancos se caracteriza por tener una gran gota lipídica que aprovecha al máximo su volumen celular, localizándose el resto de orgánulos celulares periféricamente, con citoplasma muy delgado y escasas mitocondrias[124]. Por el contrario, el TAM se localiza fundamentalmente en la zona supraclavicular y paravertebral, siendo la producción de calor su principal función[125]. Este tipo de adipocito es más pequeño que el adipocito del TAB y su citoplasma contiene varias gotitas lipídicas, un núcleo redondeado y numerosas mitocondrias que presentan en la cara interna de su membrana proteínas desacoplantes de tipo 1 (UCP1)[126] . La propiedad termogénica única de la grasa parda se debe a la presencia de las UCP1 que desacoplan la cadena respiratoria de la fosforilación oxidativa, permitiendo que los adipocitos oxiden activamente los ácidos grasos dispersándolos en forma de calor, en lugar de almacenarlos[119].

El tercer tipo de tejido adiposo se denomina beige o "brite" (*brown-in-white*) porque presenta características morfológicas y funcionales del TAM y se dispersa principalmente por los depósitos de TAB[119]. Así, los adipocitos beige se caracterizan por su morfología con gotas lipídicas multiloculares y por la presencia de mitocondrias con UCP1 que le confiere capacidad termogénica[123]. En cuanto a su origen, la teoría más asentada es la de su formación a partir de la transdiferenciación de adipocitos blancos uniloculares y de precursores mesenquimales que expresan Myf5[123] dependiendo de las demandas metabólicas o termogénicas del individuo[122,127]. No obstante, en los últimos años, varios estudios han sugerido que los adipocitos beige son funcional y molecularmente distintos de los adipocitos marrones y blancos tanto en ratones como en humanos[128,129].

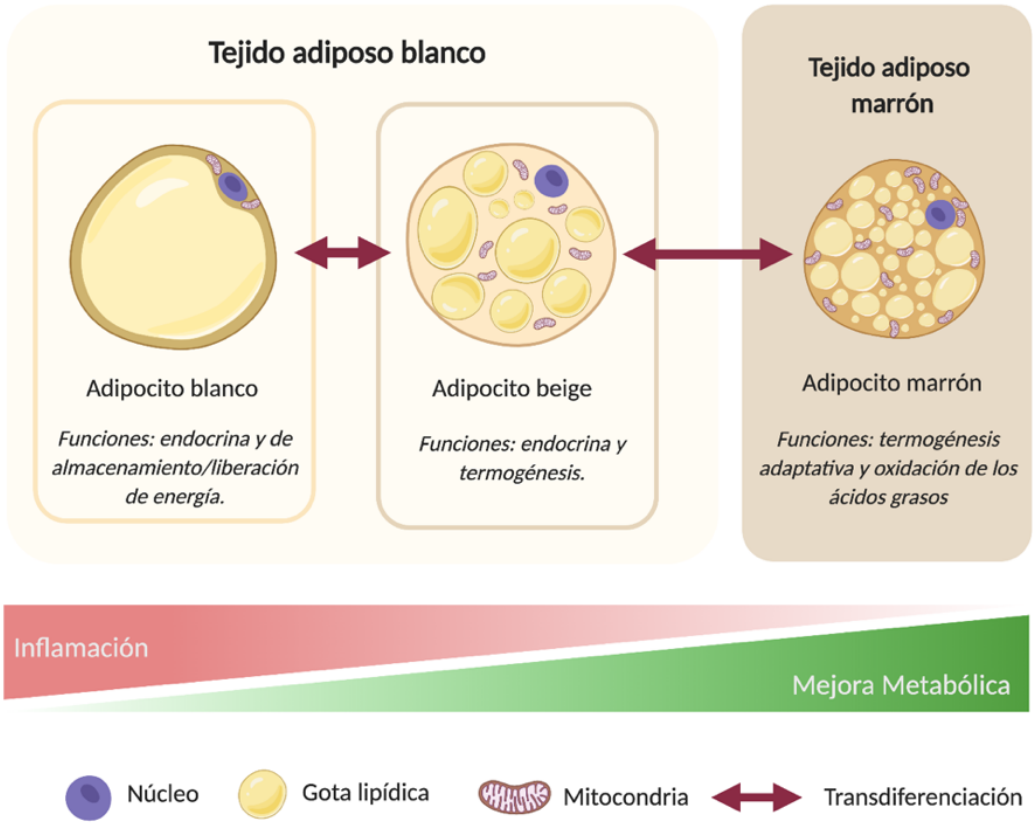


**Figura 4.** Tejido adiposo blanco y marrón: clases de adipocitos, rasgos principales de su morfología y diferencias en sus funciones celulares. Figura creada con: BioRender.com

Así, en el tejido adiposo se diferencian dos tipos de adipocitos termogénicos, los adipocitos marrones y beige, que oxidan los lípidos para producir calor. Por otra parte, encontramos los adipocitos blancos, en los que el almacenamiento ineficaz de lípidos causa la hipertrofia del TAB, lo que provoca una alteración de su funcionamiento normal, favoreciendo el desarrollo de patologías relacionadas con la obesidad.

## 3.2. CARACTERÍSTICAS DEL TEJIDO ADIPOSO BLANCO

El TAB es un órgano endocrino heterogéneo, en el que se diferencian el TAv y el TAs[119]. Estos dos tejidos, pese a tener características morfológicas similares, presentan diferencias tanto en la localización[119] y la distribución en función del sexo[130], como en la capacidad de expansión[131] y la expresión de péptidos bioactivos[132] que determinarán la funcionalidad del TA.

### 3.2.1. DISPOSICIÓN ANATÓMICA

El TAs almacena aproximadamente entre el 80-90% de la grasa corporal total, situándose debajo de las capas dérmicas, principalmente en el área abdominal, subescapular y glúteo-femoral[130]. El TAv, por el contrario, representa hasta el 10-20% de la grasa total en los hombres y el 5-8% en las mujeres[133], localizándose principalmente en los depósitos viscerales (omental y mesentérico) y, en menor medida, en áreas retroperitoneales y epicárdicas[130]. Por último, hay un pequeño porcentaje de TA que correspondería a la grasa ectópica localizada en el interior de los órganos internos (intrahepatocelular, intrapancreática, intramiocelular y intracardiomiocelular) como consecuencia de la expansión del TAv[130,134].

La disposición anatómica del TA tiene implicaciones claves en la funcionalidad de estos dos tejidos, ya que en el TAs el drenaje venoso se realiza a través de venas sistémicas mientras que el TAv (omental y mesentérico) drena directamente al hígado a través de la vena porta[133]. Este drenaje a través de la vena porta proporciona acceso hepático directo a los ácidos grasos libres y a las adipoquinas secretadas por los adipocitos viscerales, pudiendo desencadenar mecanismos inmunitarios hepáticos a través de mediadores inflamatorios como la proteína C-reactiva (PCR)[133]. Aunque son muchas las evidencias que apoyan esta hipótesis, y a día de hoy es la más aceptada, hay estudios que estiman que en personas obesas el TAs de la zona abdominal suministra la mayor parte del flujo de ácidos grasos libres a través de la vena porta y la circulación sistémica[135]. Por lo que la implicación hepática en individuos obesos podría deberse a la capacidad limitada del TAs para almacenar el exceso de energía, provocando un aumento del TAv[135]. Una investigación reciente, podría explicar estas dos hipótesis, ya que sus datos sugieren que el TAs abdominal y glúteo-femoral son depósitos con distintos perfiles de expresión y con diferente comportamiento en las complicaciones metabólicas asociadas a la obesidad[136].

### 3.2.2. DIMORFISMO SEXUAL

La cantidad, la distribución y la propia funcionalidad de los depósitos de grasa son diferentes dependiendo del sexo, especialmente después de la pubertad, debido al efecto que tienen las

hormonas esteroideas sexuales sobre el adipocito, mediando sus acciones biológicas a través de sus receptores nucleares: el receptor de estrógenos y de andrógenos[137,138].

El porcentaje de grasa corporal total es mayor en las mujeres que en los hombres[137]. Es más, antes de la pubertad, las niñas ya presentan una mayor cantidad de TAs que los niños de la misma edad [138]. Este patrón se mantiene y se acrecienta en las niñas tras la pubertad, momento en el que se produce un aumento de los estrógenos circulantes que coincide con un marcado incremento en el depósito de TAs en la región glúteo-femoral o periférica (distribución de tipo ginoide)[137]. Este tipo de disposición se va perdiendo a medida que la producción ovárica de estrógenos disminuye después de la menopausia, provocando el aumento de grasa abdominal en la mujer[139]. *Papadakis y col.* demostraron que la terapia de reemplazo con estrógenos en mujeres postmenopáusicas disminuía la masa de TAv abdominal[140]. Así, los estrógenos parecen tener un efecto protector contra el aumento de peso al acrecentar el gasto de energía y distribuir el TA en las regiones subcutáneas[141].

Los hombres presentan mayor acumulación de grasa, tanto visceral como subcutánea, en la región central (distribución de tipo androide)[137]. En la pubertad se produce un aumento de los niveles de testosterona que comienzan a disminuir a partir de los 20 años alcanzando los niveles más bajos entorno a los 70 años de edad[139]. Varios estudios en hombres han relacionado el descenso de los niveles de testosterona con un aumento de la grasa abdominal[142], provocando la restauración de los niveles fisiológicos de testosterona la disminución del TAv abdominal[143]. Un estudio en niños, concluyó que los niveles bajos de testosterona favorecían la acumulación de TAv y TAs[144].

Además de lo anteriormente descrito, en el caso de los hombres, se ha constatado una actividad positiva de los estrógenos sobre la distribución de la grasa. De tal forma que la pérdida de señalización de estrógenos en hombres promueve la obesidad[139]. En esta dirección, un estudio reciente ha demostrado que la inhibición farmacológica de los estrógenos circulantes provoca un aumento de la masa grasa, concluyendo que el estradiol es un determinante clave de la adiposidad en los hombres[142].

Si bien la acción de los estrógenos parece tener un papel beneficioso en la regulación de la grasa corporal en los hombres, en las mujeres el papel de los andrógenos parece no estar tan claro. Hay varios estudios en mujeres con síndrome de ovario poliquístico (PCOS), caracterizado por presentar altos valores de testosterona, que relacionan la presencia de este síndrome con una mayor tendencia a acumular grasa intra-abdominal[145,146]. Un metanálisis que incluyó 16 estudios independientes en adolescentes con PCOS, mostró que la presencia de obesidad se

asociaba con valores más elevados de testosterona y, que a su vez, los trastornos metabólicos en los adolescentes con PCOS empeoraban por la obesidad concomitante[147]. Otro estudio en mujeres jóvenes, puso de manifiesto que aquellas con niveles bajos de estradiol y altos de testosterona presentaban los ratios más altos de cintura-cadera; sin embargo, aquellas con valores más elevados de estradiol y testosterona mostraron los ratios cintura-cadera más bajos[148].

Por lo tanto, teniendo en cuanta que las hormonas sexuales son claves en la cantidad, distribución y funcionalidad del TA, la monitorización de sus valores sanguíneos podría resultar de utilidad en el conocimiento en la fisiopatología de la obesidad.

### 3.2.3. CAPACIDAD DE EXPANSIÓN

La capacidad de expansión del tejido adiposo es un proceso estrechamente asociado con cambios metabólicos[121] que viene determinado por la diferenciación de los preadipocitos a nuevos adipocitos (hiperplasia) o por el aumento del tamaño de los adipocitos existentes (hipertrofia)[149].

Durante la infancia y la adolescencia existen dos picos importantes de expansión del tejido adiposo por hiperplasia: el primero, tras el nacimiento, y el segundo durante el desarrollo puberal entre los 8 y 14 años[121]. Estas dos etapas se consideran críticas para el desarrollo posterior de la obesidad, ya que se ha visto que el número total de adipocitos, tanto para individuos delgados como obesos, se alcanza al finalizar la adolescencia, existiendo muy poca variación en la etapa adulta[150]. Así, durante la adolescencia la tasa de proliferación de los adipocitos disminuye y se mantiene relativamente constante en el período adulto, etapa en la que, en situaciones de exceso de peso, el TA aumentará principalmente por hipertrofia[121].

Sin embargo, estas conclusiones surgieron de estudios de obesidad de inicio temprano y actualmente se sabe que aquellas personas que en edad adulta aumentan de peso de manera prolongada en el tiempo aumentan inicialmente el tamaño de sus adipocitos hasta un cierto umbral antes de producirse el proceso de hiperplasia[117,150]. De este modo, la hiperplasia se consideraría un mecanismo de recuperación en respuesta a una sobrealimentación, provocando la ausencia de este mecanismo la aparición de complicaciones metabólicas[151].

En este contexto, son varios los estudios que diferencian entre un fenotipo de obeso “saludable” o “no saludable”[149,152] en base a la capacidad de plasticidad y funcionalidad del TA, considerándose una obesidad metabólicamente “no saludable” aquella que se asocia con hipertrofia e hipoplasia[121].

La hipertrofia es un proceso que, como consecuencia de la importante expansión de los adipocitos, conduce a la hipoxia del TA y a su depósito ectópico en órganos como el hígado, el páncreas, el corazón o el músculo esquelético[117]. Este TA hipóxico sufre pérdida de vascularización y necrosis, lo que desencadena una respuesta inflamatoria, así como la alteración de su función endocrina. Todo ello, contribuye al desarrollo de las alteraciones metabólicas asociadas a la obesidad[153]. Además, los preadipocitos, en respuesta a estímulos inflamatorios, pueden adoptar un fenotipo inflamatorio similar al de los macrófagos con una mayor expresión de citoquinas proinflamatorias y una capacidad adipogénica disminuida, incrementándose aún más la alteración de la funcionalidad del TA[149].

La hiperplasia, por el contrario, se considera un proceso beneficioso y adaptativo que mantiene la vascularización del TA y, por tanto, su funcionalidad endocrina con una correcta secreción de adipoquinas[149]. De esta forma, la hiperplasia, tanto del TA visceral como subcutáneo, se relacionaría con un efecto protector tratando de evitar la aparición de anomalías del metabolismo de los lípidos y del metabolismo hidrocarbonado en individuos obesos[154].

Es importante destacar que los dos tipos de depósitos del TA tienen capacidad de expansión diferente. El TAs tiene una tasa de crecimiento y un potencial adipogénico significativamente superior que el TAv dando lugar a adipocitos más funcionales y menos susceptibles a las respuestas inflamatorias en la obesidad[131]. Por el contrario, el TAv en estados de adiposidad excesiva presenta adipocitos hipertróficos que conduce a la liberación de mediadores proinflamatorios (TNF-α, IL-6 o leptina, entre otros)[134,155]. Varios estudios en pacientes obesos demostraron estas diferencias entre estos dos tejidos, mostrando que los pacientes obesos que presentaban expansión del TAv tenían una mayor susceptibilidad a los trastornos metabólicos, mientras que en aquellos con TAs expandido se observaban unas características metabólicamente “saludables”[134]. Para profundizar en el estudio de la funcionalidad de estos dos tejidos, en los últimos años, se han realizado experimentos de trasplante o extirpación del TAB en ratones y ratas obesas. En estos estudios se ha demostrado que la implantación del TAs era metabólicamente beneficioso, reduciendo el peso corporal y mejorando la resistencia a la insulina y la intolerancia a la glucosa y la inflamación; mientras que el TAv empeoraba la situación metabólica aumentando la intolerancia a la glucosa, la dislipidemia y el estado proinflamatorio[156], mejorando estas complicaciones metabólicas con su extirpación[157].

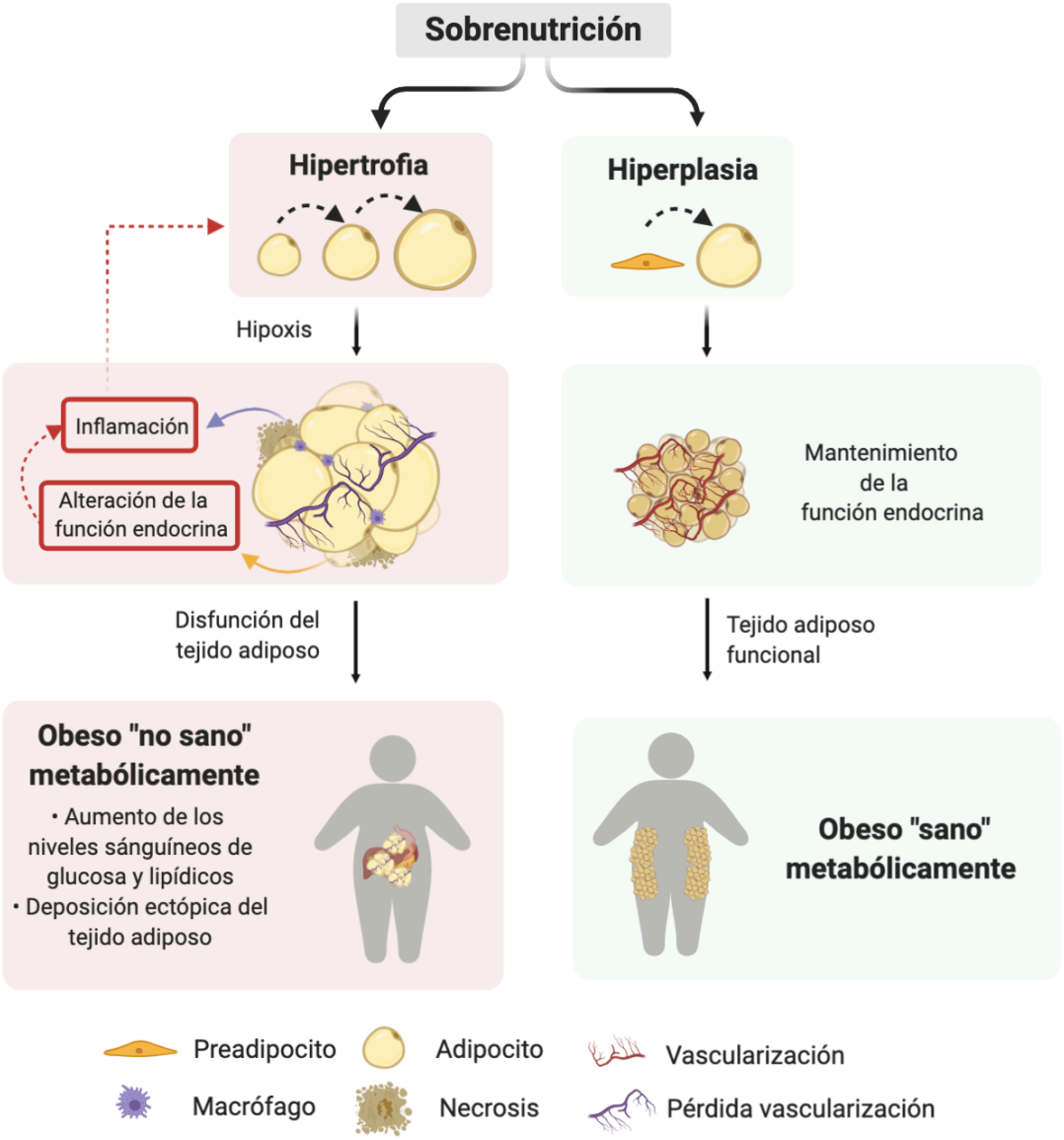


**Figura 5.** Mecanismos de expansión del tejido adiposo en estados de sobrenutrición. Figura adaptada de la revisión de *Ghaben AL y col.* [149]. Figura creada con: BioRender.com

### 3.3. Obesidad e inflamación

Tal y como acabamos de describir, la obesidad puede conducir a una disfunción grave del TAB, como consecuencia de una situación de hipertrofia e hipoxia de los adipocitos que provoca la alteración de su actividad endocrina. Las moléculas clave en la actividad endocrina del TA, las adipoquinas, son péptidos bioactivos secretados por adipocitos maduros, preadipocitos, células endoteliales y células inmunitarias del TA, con actividad autocrina, paracrina y sistémica[158].

Varios estudios sugieren que el desajuste en la producción de adipoquinas, debido a la hipertrofia del adipocito, desencadena una respuesta inflamatoria de bajo grado en el TA que, a su vez, altera todavía más la producción de adipoquinas[159]. *Shurk y col*.[160] demostraron que el

tamaño de los adipocitos constituye un factor determinante en la secreción de adipoquinas, encontrando una asociación positiva entre el tamaño de los adipocitos y la secreción de varias moléculas inflamatorias, como leptina o IL-6, y una asociación negativa con moléculas antiinflamatorias como IL-10 y adiponectina. Es más, hay trabajos que indican que la alteración de la secreción de adiponectina y leptina favorece la respuesta inflamatoria del TA, considerándose el ratio de los valores plasmáticos de estas adiopoquinas como un indicador de la disfunción del TA[161–163]. En este sentido, se ha atribuido un papel relevante en la fisiopatología del SMet a ambas adipoquinas, considerando a la leptina como una adipoquina proinflamatoria y a la adiponectina como una adipoquina antiinflamatoria[164–167].

Por tanto, la secreción normal de adipoquinas aseguraría un equilibrio "saludable" entre el TA y otros órganos, como el hígado, el cerebro, el corazón, los músculos, el sistema vascular e inmunológico; mientras que la producción anormal de adipoquinas, como ocurre en la obesidad, podría interrumpir el equilibrio homeostático y desencadenar la activación de procesos inflamatorios a nivel tisular y sistémico que provocaría el desarrollo de trastornos metabólicos y complicaciones crónicas[158] (Figura 6).

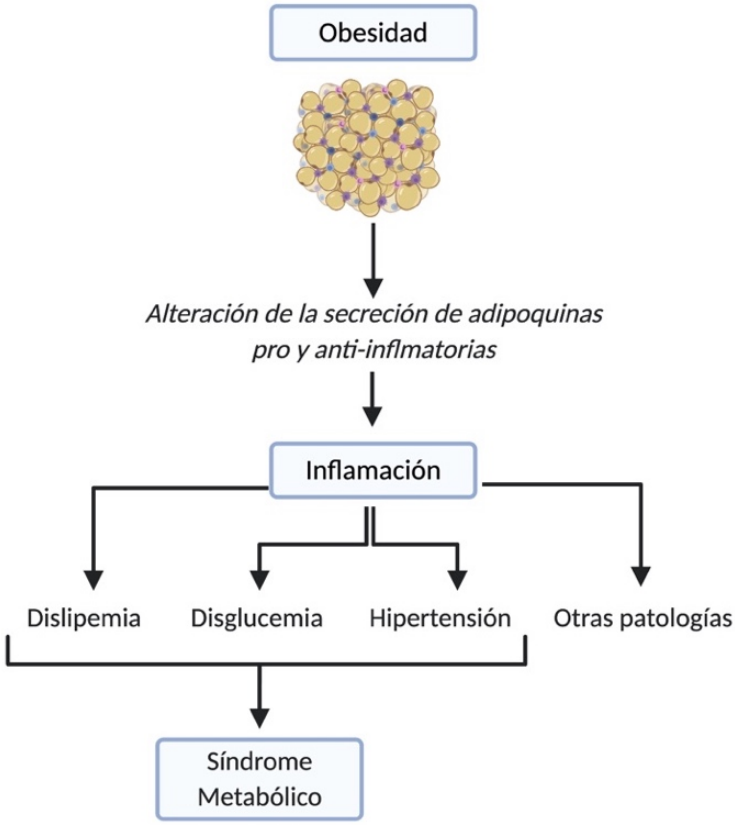


**Figura 6.** Resumen de los desordenes metabólicos causados por la desregulación de la secreción de adipoquinas en un estado de obesidad. Figura creada con: BioRender.com

Una de las moléculas implicadas en esta respuesta inflamatoria es la PCR**,** cuya síntesis hepática se desencadena, en respuesta a adipoquinas proinflamatorias secretadas por los adipocitos viscerales[133]. Son muchos los estudios que encuentran una importante asociación entre los valores plasmáticos de PCR y alteraciones metabólicas asociadas a la obesidad, como el SMet[166,168], la diabetes tipo 2 y las enfermedades cardiovasculares[169]. Además, en los últimos años, varios publicaciones han descrito una acción directa de la PCR sobre ciertas adipoquinas, como la leptina o la adiponectina[170–172], por lo que la PCR podría afectar a la propia funcionalidad de estas adipoquinas y, por tanto, al desarrollo de la obesidad y su patologías asociadas.

### 3.3.1 Proteína C-reactiva

La PCR es una proteína pentamérica dependiente de calcio[173], descubierta en 1930 en pacientes con neumonía, constituyendo la primera proteína utilizada como reactante de fase aguda[174]. Su síntesis es principalmente hepática bajo la regulación de citoquinas producidas en el sitio de la patología[173]. Tiene una vida media promedio de aproximadamente 19 horas pudiendo aumentar su concentración sanguínea hasta 1.000 veces[173]. Estas características hacen que sea uno de los marcadores de inflamación sistémica y de daño tisular inespecífico de inicio temprano (12-24 horas) más empleados en la práctica clínica[173].

Así, durante casi 60 años, únicamente se consideró a la PCR un marcador inespecífico de inflamación aguda. Sin embargo, en 1990 *Berk y col*.[175], mediante inmunoensayos con mayor sensibilidad que los previamente empleados, demostraron que los valores de PCR aumentados, incluso los que se encontraban dentro del rango antes considerado normal, se consideraban predictores de eventos coronarios futuros[173]. Este hallazgo fue el punto de partida para nuevas investigaciones produciéndose un crecimiento exponencial en el número de publicaciones sobre PCR e inflamación. A día de hoy, gracias a la sensibilidad y reproducibilidad que ha demostrado tener, la PCR es ampliamente utilizada como un marcador de enfermedad inflamatoria crónica en distintas patologías como por ejemplo la obesidad y las enfermedades cardiovasculares[176].

A lo largo de los años se ha demostrado de manera consistente, tanto en niños como en adultos, una importante asociación entre los valores sanguíneos de PCR y el IMC[169]. Nuestro propio grupo mostró, en edad pediátrica, que el exceso de peso se relacionaba con valores más elevados de PCR[177]. Además, la pérdida de peso se asoció a una disminución en los niveles de PCR. Este hallazgo nos llevó a hipotetizar que un estado inflamatorio crónico vinculado a la obesidad podría ser reversible al disminuir el IMC[177].

En los últimos años, son varios los trabajos que analizan la causalidad de esta asociación, sugiriendo que el estado de inflamación crónica no es sólo una consecuencia de la obesidad sino

que también podría resultar un factor clave en la perpetuación de este trastorno crónico[178,179]. *Piening y col.*[178], mediante técnicas de análisis ómicos, demostraron que el aumento leve de peso (2-3 kg) se asociaba directamente con la activación de señales inflamatorias. Por otra parte, aunque la pérdida de peso revertía algunos cambios, persistían una serie de señales que podrían indicar cambios fisiológicos a largo plazo. Otro estudio llevado a cabo por *Li y col*.[179] en ratas transgénicas del gen de la *PCR* de humanos, concluyó que el aumento crónico de los niveles de PCR favorecía la aparición de obesidad en el adulto. Estos autores demostraron que las ratas transgénicas presentaban de 6 a 9 veces más masa grasa (entorno al 80% grasa visceral) que las ratas control, siendo los adipocitos de este tejido tres veces más grandes que los adipocitos de las ratas de control. Estos resultados indican que el mantenimiento de valores elevados de PCR podría contribuir a la hipertrofia del adipocito**.** De esta manera, la PCR no sería un mero marcador de inflamación sino que podría tener un papel activo en el desarrollo de la obesidad[179].

Por lo tanto, resulta de gran interés el estudio no sólo de factores que puedan modular los niveles de PCR sino también profundizar en el estudio de la expresión de PCR en el tejido adiposo y su posible relación con hormonas reguladoras del metabolismo energético, como leptina y adiponectina.

### 3.3.2 Expresión de Proteína C-Reactiva en el tejido adiposo

Durante años se consideró que la PCR se producía exclusivamente en el hígado en respuesta a ciertas citoquinas, principalmente a la IL-6[173]. En el año 2003, *Ouchi y col.*[180] fueron los primeros en demostrar la expresión del gen de *PCR* en TAs humano. Estos resultados fueron confirmados por *Anty y col.*[181], quienes también demostraron expresión de *PCR* en fragmentos omentales de TA extraídos de sujetos obesos, sin encontrar diferencias significativas en la expresión de *PCR* entre las biopsias de TA subcutáneo y omental. Además, mostraron que los niveles de IL-6 podían regular la expresión de *PCR* en el propio TA y que la expresión de ambas citoquinas estaba estrechamente relacionada con la presencia de obesidad. Siguiendo esta línea de investigación, *Calabro y col.*[182] vieron que el tratamiento tanto con IL-6 como con IL-1 estimulaba la expresión de *PCR* en adipocitos humanos. Además, analizaron la posible acción que podía tener el tratamiento de adiponectina o leptina sobre *PCR*, sin encontrar ningún resultado concluyente.

Tras este estudio de *Calabro y col.* no encontramos ningún otro estudio acerca de la acción directa de leptina o adiponectina sobre la expresión de *PCR* en el TA. No obstante, esta acción es evidente en otros tejidos como el hígado o en las células endoteliales en los que la leptina puede inducir su expresión[183,184] mientras que adiponectina la inhibe[185]. A su vez, también se ha

demostrado una relación directa entre los niveles de PCR con la expresión, a nivel del adipocito, de *ADIPOQ*[170–172] y de *LEP*[171,186], y de otros genes como *TNFα, IL-6 y PPARγ*[171].

### 3.3.3 Factores reguladores de proteína C-reactiva

#### *Hormonas sexuales*

Teniendo en cuenta la importancia que presentan las hormonas sexuales en la cantidad, distribución y funcionalidad del TA, se ha señalado un papel relevante de las mismas en los procesos inflamatorios.

Recientemente, se ha analizado en una revisión el efecto antiinflamatorio de la testosterona concluyendo que el nivel de testosterona es determinante en la regulación de los procesos inflamatorios al reducir la producción de ciertas citoquinas (leptina, IL-6 o TNF-α) al mismo tiempo que estimula la producción de adiponectina[187]. Este efecto antiinflamatorio de la testosterona se refleja en los niveles de PCR, existiendo una amplia evidencia científica que encuentra una asociación negativa entre los niveles de testosterona y PCR[187,188]. Sin embargo, es importante destacar que en muchos de estos estudios, la asociación entre PCR y testosterona no se encuentra ajustada por importantes variables confundentes como el IMC[187]. Otro punto importante a destacar es que la mayoría de estos estudios se han realizado en población masculina, siendo escasos y contradictorios los llevados a cabo en población femenina[189,190]. Un estudio que incluye mujeres posmenopáusicas sin terapia hormonal sustitutiva halló una asociación negativa entre PCR y los valores sanguíneos de testosterona, sin encontrar relación significativa con los niveles de estradiol[189]; sin embargo, otro estudio en mujeres posmenopáusicas, mostró una relación positiva tanto de los niveles de estrógenos como los de andrógenos con PCR e inversa con la globulina transportadora de hormonas sexuales (SHBG)[190].

En cuanto al efecto del estradiol sobre los procesos inflamatorios en hombres, en la mayoría de los estudios no se han observado asociaciones estadísticamente significativas entre estradiol y biomarcadores inflamatorios[191], aunque algunos de ellos sí mostraron una tendencia positiva[192–194]. Esta tendencia se vio en el estudio de *Tsilidis y col.*[195] mostrando una relación positiva significativa entre los niveles de estradiol y PCR, incrementándose la probabilidad de tener niveles elevados de PCR (≥3 mg/L) con valores altos de estradiol, tanto libre como total.

En mujeres, si bien es cierto que hay muchos estudios que documentan un efecto protector de los estrógenos contra el aumento de peso[141], son escasos los estudios que evalúan su posible relación con PCR. La mayoría de las evidencias proceden de estudios en mujeres posmenopáusicas tratadas con terapia hormonal sustitutiva. En mujeres premenopaúsicas, en las que aún hay menos evidencias, se observó que durante el ciclo menstrual se producía un

aumento de los niveles de estradiol que se asociaba significativamente con un descenso de los niveles de PCR[196–198]. Por último, un estudio reciente ha confirmado una diferente asociación entre los niveles de estradiol y PCR teniendo en cuenta la etapa de fertilidad de la mujer. Así, los niveles séricos de estradiol estaban inversamente asociados con los niveles de PCR en mujeres premenopaúsicas, pero no en mujeres posmenopaúsicas[199].

En edad pediátrica, también se ha descrito una asociación entre los niveles de PCR con los esteroides sexuales y con SHBG (Tabla 2). En niños, el estudio de *Mogri y col.*[200] encontró una asociación negativa ente los niveles de testosterona libre y total con PCR. Estos mismos resultados se observaron en un estudio longitudinal en niñas en las que se vio una asociación negativa entre testosterona total y PCR que desapareció después de la menarquía[201]. En cuanto al estradiol, únicamente encontramos un estudio en niños y niñas turcos, en los que se concluyó que el índice de estradiol libre es un importante predictor de los niveles de PCR en niños, mientras que en niñas únicamente sería el IMC[202]. Por último, dos investigaciones mostraron una asociación negativa entre los niveles de SHBG y PCR tanto en niños como en niñas[203,204]. Por lo que los niveles de las hormonas sexuales podrían postularse como un factor regulador de los niveles de PCR a tener en cuenta ya en edad pediátrica.

**Tabla 2.** Resumen de estudios publicados que han analizado la posible asociación de hormonas sexuales y niveles de proteína C-reactiva en población pediátrica.

| Autores (Ref) | Sujetos (n) | Edad | Marcador de inflamación |
|---|---|---|---|
| *Sørensen y col.*[203] | 62 ♂ / 70 ♀ | 8.5–16.1 | Asociación negativa entre SHBG y PCR en niños y niñas. |
| *Mogri y col.*[200] | 50 ♂ | 14-20 | Asociación negativa entre los niveles de T libre y PCR. Resultados similares con T total. |
| *Pinkney y col.*[204] | 375 ♂ / ♀ | 5-15 | Asociación negativa entre SHBG y PCR en niños y niñas. |
| *Wen y col.*[201] | 396 ♀ | 11.2±0.8 | Asociación negativa entre T total y PCR antes de la menarquia que se pierde después de la menarquia. |
| *Ağırbaşlı y col.*[202] | 91 ♂ /77 ♀ | 8-17 | El FEI es predictor de los niveles de PCR en niños. |

♂: niños; ♀: niñas; PCR: proteína C-reactiva; SHBG: globulina fijadora de hormonas sexuales; T: testosterona; FEI: índice de estradiol libre (Free Estradiol Index).

### *DIETA Y ANTIOXIDANTE PLASMÁTICOS*

La inflamación crónica de bajo grado es una de las características distintivas de la enfermedad metabólica inducida por la dieta[149]. Los factores dietéticos, como los ácidos grasos y los antioxidantes modulan potencialmente la asociación entre la adiposidad y la inflamación subclínica pudiendo influir en la expresión de ciertas citoquinas proinflamatorias[149].

*Barbaresko y co*l.[205] analizaron un total de 46 estudios sobre patrones alimentarios y biomarcadores de inflamación, concluyendo que los patrones basados en la carne se asociaban de forma estadísticamente significativa con biomarcadores proinflamatorios como la PCR, la IL-6 y el fibrinógeno. Mientras que los patrones basados en vegetales y frutas o los denominados patrones "saludables" tendían a estar inversamente relacionados con los biomarcadores inflamatorios. Así, destacan los estudios de intervención que investigaron la dieta mediterránea y que mostraron una importante consistencia en los resultados señalando a este tipo de dieta un efecto antiinflamatorio[205].

Hasta la fecha, nuestro grupo ha investigado la relación entre dieta y PCR tanto en población adulta como en población pediátrica. En población adulta, en el estudio SPREDIA-2, encontramos que la adherencia a una dieta de tipo mediterránea se asociaba con valores sanguíneos más bajos de PCR[206]. En concreto, constatamos que esta asociación parecía estar particularmente relacionada con un mayor consumo de verduras, frutas, productos lácteos y pescado[206]. En población infantil, gracias al estudio 4P, concluimos que factores dietéticos específicos, como la ingesta de grasas saturadas, frutas, verduras y fibra, y el contenido en vitamina A y E, se relacionaban con variaciones en los niveles de PCR en niñas, independientemente del IMC[207].

En este contexto, varios estudios mostraron que las concentraciones plasmáticas de ciertas vitaminas liposolubles, como la vitamina A (retinol, carotenoides y licopenos) y la vitamina E (tocoferoles), disminuían durante la respuesta inflamatoria de fase aguda se asociaba con un aumento de marcadores inflamatorios, como la PCR[208,209]. Un metanálisis de ensayos controlados aleatorios que analiza el efecto de la suplementación con vitamina E sugiere que la suplementación con α-tocoferol o γ-tocoferol reduce las concentraciones de PCR en sangre[210]. En esta línea, *Schwab ycol.*[211] demostraron que la vitamina E en combinación con la ingesta de otros antioxidantes, especialmente la vitamina C, disminuía los niveles de PCR.

Hasta la fecha, los estudios que han analizado la asociación de distintas vitaminas plasmáticas con los niveles de PCR en adultos han mostrado diversas evidencias (Tabla 3). En algunos de ellos, las concentraciones de PCR se han relacionado con niveles más bajos de carotenos (α-caroteno, β-caroteno)[212–215], vitamina A (retinol)[214,216,217] y licopeno[212]; mientras que otros no han logrado encontrar una asociación entre los niveles sanguíneos de PCR con vitamina E total[214] o con α-tocoferol [218], o han encontrado una asociación positiva entre PCR y α-tocoferol [218] o γ-tocoferol[217]. Por otra parte, es importante apuntar que se han documentado diferencias raciales en la relación de las vitaminas A, E y β-caroteno con la PCR[219].

**Tabla 3.** Resumen de los principales estudios publicados en adultos que han analizado la posible asociación de vitamina A y E y niveles de proteína C-reactiva.

| Autores (Ref) | Sujetos (n) | Estudio | Edad | Vitaminas | Asociación con PCR |
|---|---|---|---|---|---|
| *Kritchevsky col.* [212] | 3180 ♂/♀ | NHANES III | 20-25 | α-caroteno | Asociación negativa |
| | | | | β-caroteno | Asociación negativa |
| | | | | Licopeno | Asociación negativa |
| *Erlinger y col.* [213] | 14470 ♂/♀ | NHANES III | ≥ 18 | β-caroteno | Asociación negativa |
| *Ford y col.*[214] | 3213 ♂/♀ | NHANES III | ≥20 | Retinol | Asociación negativa |
| | | | | Vitamina E | Sin cambios significativos |
| | | | | α-caroteno | Asociación negativa |
| | | | | β-caroteno | Asociación negativa |
| | | | | Licopeno | Asociación negativa |
| *Il'Yasova y col.*[215] | 60 ♂/♀ | TRAIN | 55-81 | β-caroteno | Asociación negativa |
| *Wang y col.*[224] | 2878 ♀ | WHS | ≥45 | α-caroteno | Asociación negativa |
| | | | | β-caroteno | Asociación negativa |
| | | | | Licopeno | Sin cambios significativos |
| *Wood y col.*[217] | 2010 ♀ | APOS | 55-66 | Retinol | Asociación negativa |
| | | | | α -tocoferol | Sin cambios significativos |
| | | | | γ-tocoferol | Asociación positiva |

♂: hombres; ♀: mujeres ; PCR: proteína C-reactiva; NHANES III: *Third National Health and Nutrition Examination Survey* (Tercera Encuesta Nacional de Examen de Salud y Nutrición); WHS: *Women's Health Study* (Estudio de salud de la mujer); TRAIN: *Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors* (Ensayo de inhibición de la enzima convertidora de angiotensina y nuevos factores de riesgo cardiovascular); APOS: *Prospective Osteoporosis Screening Study* (Estudio prospectivo de detección de osteoporosis)

Los estudios que analizan la asociación entre la PCR y los niveles de antioxidantes en niños son escasos y se ha investigado principalmente en niños con diversas patologías como enfermedades infecciosas[220], ceguera[221] u obesidad[222], encontrándose en todos estos estudios una asociación negativa entre los niveles de PCR y vitamina A. Uno de los estudios internacionales más importantes llevados a cabo en población infantil es el de *Stephensen y Gildengorin*[223], en el que también encontraron una asociación negativa entre los niveles de PCR y el retinol.

Hasta donde sabemos, no se ha realizado un estudio transversal que analice conjuntamente la asociación de la PCR con los valores sanguíneos de las vitaminas liposolubles antioxidantes A y E en niños sanos.

## HIPÓTESIS

La obesidad, caracterizada por un exceso de tejido adiposo, es el resultado de un desequilibrio energético entre ingesta y gasto calórico a lo largo del tiempo. A este respecto, el cerebro juega un papel clave en el control de la homeostasis energética, respondiendo tanto a señales centrales como periféricas. Entre estas señales, se considera que desempeñan un papel fundamental ciertas hormonas sintetizadas en el tejido adiposo como la leptina. Sin embargo, evidencias científicas apuntan a que la insulina podría condicionar la acción de la leptina, y que la acción de péptidos recientemente descubiertos, como nesfatina-1 y adropina, podrían contribuir, asimismo, a explicar el complejo mecanismo de regulación relacionado con la homeostasis de la energía, el control del metabolismo de la glucosa y de los lípidos. Por otra parte, la alteración de la funcionalidad del tejido adiposo asociada a la obesidad, además de provocar una alteración de su función endocrina (cambios en la síntesis y secreción de leptina y adiponectina, entre otras), desencadenaría una respuesta inmune que provocaría un estado de inflamación crónica de bajo grado reflejado en niveles circulantes de proteína C-reactiva (PCR) elevados. En este contexto, la PCR podría no ser únicamente un marcador de inflamación de síntesis hepática, sino que podría ser sintetizada también por el tejido adiposo, pudiendo participar en la etiopatogenia de la alteración de la funcionalidad del tejido adiposo y contribuir al desarrollo de comorbilidades asociadas a la obesidad, de forma independiente a la acción de las adipoquinas.

## OBJETIVOS

1. **Profundizar en el estudio, en población pediátrica, de diferentes péptidos implicados en la regulación de la homeostasis de la energía.**
    1.1. Estudiar la interacción de leptina e insulina en la regulación de la ingesta dietética en la primera cohorte del estudio 4P (niños de 6 a 8 años).
    1.2. Investigar el papel que desempeñan otros péptidos de secreción central y periférica en la homeostasis de la energía en ambas cohortes del estudio 4P (niños de 6 a 8 años y adolescentes de 12 a 16 años) .
        1.2.1. Evaluar en un estudio caso-control la posible asociación entre los niveles plasmáticos de nesfatina-1 y la presencia de obesidad.
        1.2.2. Analizar en un estudio caso-control la posible relación entre los niveles plasmáticos de adropina y la presencia de obesidad.

2. **Estudiar el papel de proteína C-reactiva (PCR), en población pediátrica, como marcador de inflamación crónica y de alteraciones metabólicas e investigar los factores relacionados con sus niveles plasmáticos.**
    2.1. Investigar la posible expresión de PCR en tejido adiposo en población pediátrica sometida a una apendectomía.
    2.2. Evaluar la utilidad de PCR como biomarcador de síndrome metabólico independiente de los niveles de leptina y adiponectina, en la cohorte de adolescentes del estudio 4P.
    2.3. Determinar posibles factores relacionados con los niveles plasmáticos de PCR.
        2.3.1 Investigar la posible asociación entre los valores sanguíneos de PCR y los niveles de hormonas sexuales en la segunda cohorte del estudio 4P.
        2.3.2 Estudiar la relación de las concentraciones de PCR con los niveles de los niveles de vitaminas plasmáticas en la primera cohorte del estudio 4P.

# OBJETIVO 1:
# PROFUNDIZAR EN EL ESTUDIO, EN POBLACIÓN PEDIÁTRICA, DE DIFERENTES PÉPTIDOS IMPLICADOS EN LA REGULACIÓN DE LA HOMEOSTASIS DE LA ENERGÍA

## OBJETIVO 1.1. ESTUDIAR LA INTERACCIÓN DE LEPTINA E INSULINA EN LA REGULACIÓN DE LA INGESTA DIETÉTICA EN LA PRIMERA COHORTE DEL ESTUDIO 4P (NIÑOS DE 6 A 8 AÑOS).

Referencia:

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### RESUMEN

Antecedentes y objetivos: La asociación entre las concentraciones de leptina y la ingesta dietética resulta paradójica en los estudios en humanos. La posible existencia de una acción conjunta de leptina e insulina en la regulación de la homeostasis energética podría contribuir a explicar estas discrepancias. Nuestro estudio tuvo como objetivo evaluar la asociación entre la leptina y la ingesta dietética en función de los niveles de insulina en una cohorte de niños sanos.

Métodos y resultados: El estudio incluyó a 747 niños de 6 a 8 años (372 niños y 375 niñas). Las concentraciones de leptina se determinaron mediante un ensayo de inmunoabsorción ligado a enzimas (ELISA). Los niveles de insulina y sulfato de dehidroepiandrosterona (DHEA-S) se midieron mediante radioinmunoensayo. La información sobre la ingesta de energía y nutrientes se obtuvo mediante un cuestionario de frecuencia alimentaria. El análisis de correlación de Pearson no mostró correlaciones significativas entre la leptina y la ingesta dietética, ni en niños ni en niñas. Las correlaciones permanecieron no significativas después de ajustar por índice de masa corporal (IMC). Sin embargo, en las niñas, después de ajustar por los niveles de insulina, observamos correlaciones negativas significativas ($p < 0,01$) entre las concentraciones de leptina y la ingesta diaria de energía, grasas y carbohidratos. En las niñas con niveles más bajos de insulina, hubo una disminución significativa ($p<0,05$) en la ingesta de energía en aquellas que tenían niveles más altos de leptina; mientras que las niñas que presentaban niveles más altos de insulina no hubo cambios significativos en la ingesta de energía dependiendo de los niveles de leptina. En los niños no se encontraron tales asociaciones.

Conclusiones: Los resultados de nuestro estudio en niños de 6 a 8 años muestran que la relación de la leptina con la ingesta dietética es evidente solo después de ajustar por los niveles de insulina en niñas en las que los niveles de leptina son significativamente más altos que en los niños, lo que apoya la acción conjunta de leptina e insulina en la regulación de la homeostasis energética.

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ORIGINAL ARTICLE

# Effect of leptin and insulin concentrations on dietary energy intake in children

Olaya de Dios[1], Asha Jois[1, a], Leticia Herrero[1], Claudia Vales-Villamarín[1], Teresa Gavela-Pérez[2], Lydia Gorgojo[3], José M. Martín-Moreno[3], Leandro Soriano-Guillén[2] and Carmen Garcés[1*]

[1] Lipid Research Laboratory, IIS-Fundación Jiménez Díaz, 28040 Madrid, Spain;
olaya.dios@quironsalud.es (OdD);
ashamaya08@gmail.com (AJ);
leticia.herrerot@quironsalud.es (LH);
claudia.vales@quironsalud.es (CV-V)
[2] Department of Pediatrics, IIS-Fundación Jiménez Díaz, 28040 Madrid, Spain; TGavela@fjd.es (TG-P); LSoriano@fjd.es (LS-G).
[3] Department of Preventive Medicine & INCLIVA, University of Valencia, 46010 Valencia, Spain;
lydia.gorgojo@gmail.com (LG.);
dr.martinmoreno@gmail.com (JMM-M)
[a] Permanent address: Royal Children's Hospital, Melbourne, Australia.

**Corresponding author:**
Dr. Carmen Garcés
Lipid Research Laboratory, IIS-Fundación Jiménez Díaz
Avda. Reyes Católicos, 2. 28040 Madrid, Spain
Telephone: +34-91 5404892
cgarces@fjd.es

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leptin, insulin, dietary energy intake, children, gender.

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## Abstract

**Background and Aims:** The association between leptin concentrations and dietary intake appears paradoxical in human studies. The suggested existence of integrative actions of leptin and insulin signaling in the regulation of energy homeostasis could contribute to explain these discrepancies. Our study aimed to evaluate the association between leptin and dietary intake depending on insulin levels in a cohort of healthy children.

**Methods and Results:** The study included 747 6-to–8- year-old children (372 boys and 375 girls). Leptin concentrations were determined by enzyme-linked immunosorbent assay (ELISA). Insulin and dehydroepiandrosterone sulfate (DHEA-S) levels were measured by radioimmunoassay. Information on energy and nutrient intakes were obtained through a food-frequency questionnaire. Pearson Correlation analysis showed no significant correlations between leptin and dietary intake, either in boys or girls. Correlations remained not significant after adjusting by body mass index. However, in girls, after adjusting by insulin levels, we observed significant negative correlations ($p<0.01$) between leptin concentrations and daily energy, fat and carbohydrate intakes. Among girls with lower insulin levels, there was a significant ($p<0.05$) decrease in energy intake across leptin tertiles that was not significant in girls with higher insulin levels. No such associations were found in boys.

**Conclusions:** The results of our study in 6- to 8-year-old children show that the relationship of leptin with dietary intake is evident only after controlling by insulin levels in girls in whom leptin levels are significantly higher than in boys which supports the overlapping functions of leptin and insulin signals in the regulation of energy homeostasis.

## 1. Introduction

Obesity is the result of an energy imbalance where energy intake exceeds caloric expenditure over time, leading to an increase in body mass. The brain plays a key role in the control of energy balance, responding to peripheral signals that report nutritional status[1].

Leptin and insulin are considered two of these key peripheral signals in energy homeostasis that act in the hypothalamus and other areas of the brain[2]. Leptin is an adipokine secreted mainly by the white adipose tissue with levels directly related to the total amount of body fat, while insulin is secreted by the pancreatic β-cells dependent on blood glucose and adiposity levels. Both molecules, leptin and insulin, can enter the brain through the blood-brain barrier and act on their respective receptors on areas of intake control, specifically on the arcuate nucleus, activating the secretion of anorexigenic neuropeptides and inhibiting the expression of orexigenic neuropeptides[3].

The anorexic action of each of these two signals separately has been widely demonstrated. Studies in animal models have shown that intracerebroventricular administration of leptin and insulin reduces food intake and body weight in mice[4]. The anorexigenic effects of these hormones are mediated by leptin and insulin receptors expressed in the central nervous system[5,6]. The role of the brain insulin receptor in controlling body weight has been demonstrated animal models[7–9]. In humans, congenital deficiency of leptin has been associated with morbid obesity[10] and excessive eating[11].

When analyzing both signals in the regulation of food intake and body weight simultaneously, Air et al. [12] demonstrated that, in rats, the co-administration of leptin and insulin at the intracerebroventricular level decreased food intake and weight in an additive way. Subsequently, it has been shown that, in some hypothalamic areas, insulin and leptin activate overlapping enzymatic cascades, and that the anorexigenic action of each could inhibit or stimulate the other[13]. Thus, the evidence of cross-talk between leptin and insulin at a central level has been suggested[14].

The effect of leptin and insulin on each other's action could contribute to explain the conflicting results yielded from population studies regarding the association of leptin levels[15–24] with energy intake, with studies finding positive associations, negative association or failing to find any significant relationship.

According to our knowledge, no study analyzing this association has taken into account the joint action of leptin and insulin on the regulation of energy intake. Therefore, our study aimed to examine the influence of insulin levels on the relationship of leptin levels with dietary intake in a representative sample of healthy 6-to 8-year-old Spanish children.

## 2. Methods

### 1.1. Subjects:

Our study included a sub-population of 6- to 8-year-old children who were participants in a cross-sectional study designed to analyze cardiovascular risk factors in Spanish schoolchildren, the Four Provinces Study (4P Study). The design of the 4P Study has been described in detail previously[25]. In this study, the population comprises 747 of those children in whom information on leptin and insulin concentrations and anthropometric and nutritional data was available.

Parents or legal guardians were required to sign a written consent form allowing their children to participate in the study. The study protocol was approved by the Ethics Committee of Clinical Investigation of the Instituto de Investigación Sanitaria-Fundación Jiménez Díaz (PIC016-2019 FJD), Madrid, Spain. The investigation fulfills the principles contained in the Declaration of Helsinki and subsequent reviews, as well as the prevailing Spanish legislation on clinical research in human subjects.

#### 1.2. Anthropometric data:

Weight and height were taken with children wearing light clothing and barefoot. Height was measured to the millimeter using a portable stadiometer, and weight was recorded to the nearest 0.1kg using a standard electronic digital scale. Body mass index (BMI) (weight in kilograms divided by height in meters squared, kg/m2) was calculated from these parameters. Z-score BMI was calculated according to Spanish reference data.

#### 1.3. Biochemical data:

Blood samples were collected in the morning after overnight fasting. Insulin was measured in serum by radioimmunoassay using a commercial kit (BI-Insulin IRMA; Bio-Rad, France). Leptin concentration was measured in plasma using a commercial human leptin ELISA kit (EIA-2395, DRG, Germany), according to manufacturer's protocol. The functional sensitivity for the leptin assay was 0.7 ng/ml, and the intra and inter-assay coefficients of variation were 2.0% and 8.5%, respectively. Plasma DHEA-S was determined by RIA, using a commercial kit (DHEA-S RIA, DSL, Texas, USA).

#### 1.4. Dietary data:

Information on nutrient intake was obtained through a food-frequency questionnaire (FFQ) initially developed for use on adults and previously validated in Spain by Martín-Moreno and cols[26]. For the purpose of the 4P study, the questionnaire was adapted to a primary school population by amending and downscaling the list of foods and portions consumed on the basis of a systematic review of child-population food surveys in Spain. Full details of the dietary assessment have been reported in detail previously[27].

#### 1.5. Statistical analysis:

The characteristics of participants are summarized as mean (SD). All further analyses were carried out for boys and girls separately due to their different leptin levels and different nutritional data. The normality of the distribution of the variables under study was examined using the Kolmogorov–Smirnov test. Variables that were not normally distributed were log-transformed before analysis. When data log-transformation failed to produce normal distribution (BMI and BMI z-score), non-parametric tests were used. Characteristics for boys and girls were compared by the Student's t-test and Mann-Whitney tests depending on the distribution of the data. The associations between dietary intake, leptin and insulin concentrations were evaluated by Pearson correlation analysis. Partial correlation analysis between dietary intake and leptin was evaluated adjusting for BMI and for BMI and insulin. Crude and

adjusted linear regression analysis between leptin and food intake by sex was also carried out. ANOVA test and the corresponding Post Hoc test were used to compare the dietary intake by tertiles of leptin levels depending on groups of insulin levels. In this analysis, tertiles of leptin were calculated for each sex and insulin levels were categorized according to the median value (3 µUI/ml). Differences in leptin levels between low and high energy intake groups in males and females, adjusted by insulin, were investigated by analysis of covariance (ANCOVA). Statistical analysis was performed using the SPSS software package, version 25.0 (Chicago, Illinois), and GraphPad Prism statistical software, version 8 (San Diego, California).

## 2. Results

The characteristics (age, anthropometric and biochemical parameters, and nutritional data) of the 747 children (372 boys and 375 girls) included in our study are summarized in Table 1. Age, BMI, BMI z-score, prevalence of overweight, insulin and DHEAS were not different between sexes. Leptin levels were significantly higher in girls than in boys (Table 1). Total energy intake and protein, fat and carbohydrate intakes were significantly higher in boys than in girls (Table 1).

**Table 1.** Characteristics (mean (SD)) of the study participants

| | Boys (n=372) | Girls (n=375) | p-value |
|---|---|---|---|
| Age (years) | 7.02 (0.60) | 7.01 (0.61) | NS |
| BMI (kg/m2)[a] | 16.9 (2.4) | 16.9 (2.5) | NS |
| BMI z-score [a] | 0.02 (0.99) | 0.04 (1.00) | NS |
| Overweight prevalence (%)[b] | 23 | 28.8 | NS |
| Leptin (ng/ml) | 4.1 (5.1) | 6.4 (6.6) | <0.001 |
| Fasting insulin (µUI/ml) | 3.4 (2.2) | 3.6 (2.5) | NS |
| DHEAS (µg/dl) | 37.4 (39.3) | 36.7 (35.1) | NS |
| Energy intake (kcal/day) | 2 201 (528) | 2 044 (530) | <0.001 |
| Proteins (g/day) | 95.0 (26.3) | 89.8 (26.6) | <0.001 |
| Total fat (g/day) | 111.0 (25.7) | 105.5 (28.1) | <0.001 |
| Carbohydrate (g/day) | 213.4 (68.5) | 198.6 (63.0) | 0.001 |

p-value: T-test analysis of log-transformed variables. [a] Mann-Whitney test. [b]Chi- squared test. Abbreviation: BMI, body mass index; DHEAS, dehydroepiandrosterone-sulphate

Leptin and insulin concentrations were strongly correlated in both sexes, with correlation coefficients of 0.436 (p<0.001) and 0.513 (p<0.001) for boys and girls respectively. These associations remained significant after adjusting for BMI in both boys (r=0.268, p<0.01) and girls (r=0.392, p<0.001).

Correlation coefficients between leptin concentrations, BMI and dietary intake by sex are shown in Table 2. Pearson correlation analysis showed no significant correlations between leptin and dietary intake, either in boys or girls (Table 2). Correlations remained not statistically significant after adjusting by BMI. However, after adjusting for BMI+insulin, we observed negative significant correlations between leptin concentration and total daily energy intake (r=-0.160; p=0.003), total fat (r=-0.159; p=0.003) and total carbohydrate (r=-0.157; p=0.006) in girls (Table 2). No significant correlations were found in boys.

**Table 2.** Correlation analysis between leptin and BMI and dietary intake.

| | Pearson correlation coefficients | | Adjusted by BMI | | Adjusted by BMI + Insulin | |
|---|---|---|---|---|---|---|
| | Boys | Girls | Boys | Girls | Boys | Girls |
| BMI | 0.688** | 0.712** | | | | |
| BMI z-score | 0.686** | 0.699** | | | | |
| Energy intake | 0.037 | -0.061 | -0.027 | -0.100 | -0.024 | -0.160* |
| Protein | -0.005 | -0.024 | -0.033 | -0.041 | -0.028 | -0.079 |
| Total fat | 0.019 | -0.046 | -0.02 | -0.088 | -0.024 | -0.159* |
| Carbohydrate | 0.074 | -0.072 | -0.015 | -0.098 | -0.008 | -0.157* |

*p<0.01; **p<0.001

The results of the correlation analysis were confirmed by comparing the results of a crude lineal regression analysis between leptin levels and food intake with the results of a multivariate linear regression analysis including leptin and insulin as independent variables (Table 3).

To further evaluate the influence of insulin on the association between energy intake and leptin concentrations, we compared mean energy intake between leptin tertiles in boys and girls classified in two groups according to their insulin levels: low insulin levels (insulin less than or equal to 3 µUI/ml) and high insulin levels (insulin higher than 3 µUI/ml) (Figure 1). In boys, no significant differences were observed between leptin tertiles in either of the two groups of insulin levels (Figure 1a). In girls, we observed a significant decrease in mean energy intake across leptin tertiles among those with insulin levels ≤ 3 µUI/ml (ANOVA, p=0.018), but no significant differences in energy intake between leptin tertiles were observed in the group with higher insulin levels (Figure 1b).

An additional analysis was performed to investigate the influence of insulin on the relationship between leptin levels and energy intake categorizing energy consumption in low and high consumption according to the median value (2122 and 2014 kcal/day in boys and girls respectively) and comparing leptin levels by intake group with or without insulin adjustment. No significant differences in leptin levels by intake group were observed in the unadjusted analysis and significant differences between groups appear in girls in an ANCOVA after adjusting by insulin (Table 4).

## 3. Discussion

Based on evidences suggesting the integrative signaling of leptin and insulin in the regulation of energy homeostasis in the brain[14], we investigated if an interaction between leptin and insulin could explain energy intake in healthy children. Our study, indeed, failed to find any significant association when analyzing the direct relationship of leptin levels with dietary intake in a cohort of 6- to 8-year-old boys and girls, independently of BMI, but found a significant negative association of leptin concentrations with total energy intake and fat and carbohydrate intakes when adjusting by insulin levels. However, these associations

**Table 3.** Crude and adjusted linear regression analysis between leptin and food intake by sex

| | Boys | | | |
|---|---|---|---|---|
| | Crude $\beta$ (95% CI) | p-value | *Adjusted $\beta$ (95% CI) | p-value |
| Energy intake | 0.037 (-0.017 to 0.036) | NS | 0.032 (-0.021 to 0.038) | NS |
| Proteins | -0.005 (-0.033 to 0.030) | NS | -0.004 (-0.036 to 0.034) | NS |
| Total fat | 0.019 (-0.021 to 0.030) | NS | 0.028 (-0.030 to 0.049) | NS |
| Carbohydrate | 0.074 (-0.010 to 0.061) | NS | 0.017 (-0.046 to 0.062) | NS |
| | **Girls** | | | |
| | Crude $\beta$ (95% CI) | p-value | *Adjusted $\beta$ (95% CI) | p-value |
| Energy intake | - 0.061 (-0.043 to 0.011) | NS | -0.148 (-0.070 to -0.008) | 0.014 |
| Proteins | -0.024 (-0.039 to 0.024) | NS | -0.066 (-0.058 to 0.016) | NS |
| Total fat) | -0.046 (-0.040 to 0.015) | NS | -0.148 (-0.072 to -0.009) | 0.013 |
| Carbohydrate | -0.072 (-0.058 to 0.010) | NS | -0.143 (-0.087 to -0.008) | 0.017 |

*Adjusted by insulin levels. $\beta$: regression coefficient; CI: confidence interval.

were observed in girls but not in boys, which could be related to the higher levels of leptin in girls. The anorexigenic role of leptin is evident in girls with low insulin levels. When insulin levels are higher, leptin sensitivity related to dietary intake appears to be lower.

**Table 4.** Leptin levels (mean (SE)) by energy intake by sex unadjusted and insulin adjusted

| | Boys | | | Girls | | |
|---|---|---|---|---|---|---|
| | Energy intake | | | Energy intake | | |
| | Low | High | p-value | Low | High | p-value |
| Unadjusted | 4.0 (0.4) | 4.1 (0.4) | 0.826 | 6.3 (0.4) | 6.2 (0.5) | 0.779 |
| Adjusted by insulin | 3.9 (0.3) | 4.1 (0.3) | 0.664 | 6.6 (0.4) | 6.1 (0.4) | 0.018 |

Population studies analyzing the association of leptin concentrations with dietary intake have yielded differing results. Similar to our results, leptin levels were negatively correlated to total energy intake and carbohydrate and fat intakes in healthy postmenopausal women[16]. Yannakoulia et al.[18] also found a negative association between leptin concentrations and dietary intake in 14- to 26-year-old males and females analyzed together. However, a study including only young females failed to find any independent association between serum leptin concentration and protein, fat or carbohydrate intake[20], and a negative correlation between leptin levels and energy intake after normalizing for fat mass has been described in males but not in females in the study of Miller et al.[17].

Ostlund et al. [15] found no association between plasma leptin concentration and energy intake during a weight-maintenance diet in normal weight and subject with obesity in a wide age range (18 to 90 years). Furthermore, the INTERLIPID Study reported differing associations between leptin concentrations and dietary intake depending on weight category[21]. Therefore, the results of all these studies seem to indicate that the association between leptin concentrations and dietary intake is different depending on factors such as sex, age, weight category or hormonal status. Ostlund et al.[15] suggest that the leptin signaling system may be altered with age similar to other endocrine systems and that subjects with obesity may develop a diminished response in the leptin receptor signaling pathway or poor penetration of the blood-brain barrier by leptin[15].

Our data, showing an influence of basal insulin levels on the association between leptin and dietary intake may contribute to explain these different results. Leptin and insulin are adiposity signals[28,29] with overlapping physiological functions regulating energy homeostasis as both enter the brain and act in the hypothalamic areas involved in the regulation of feeding behavior[30]. The interaction between leptin and insulin has been shown in several studies. The observed long-term effect of insulin on leptin production, both in vivo and in vitro, suggests that insulin regulates ob gene expression and leptin production[31]. Furthermore, it has been demonstrated that insulin resistance can alter leptin signaling in a hypothalamic cell line[32].

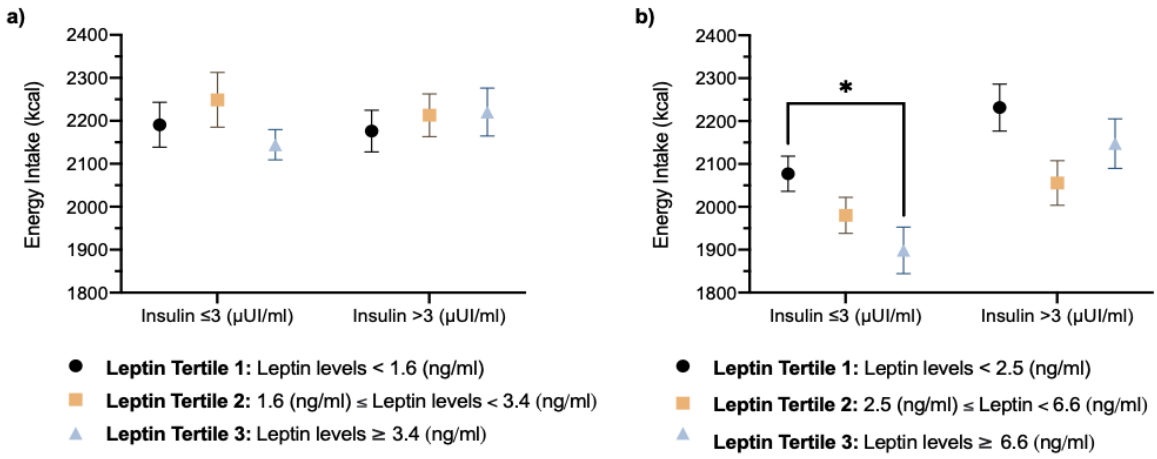


**Fig. 1** Energy intake (kcal) by tertiles of leptin levels depending on insulin levels in boys (a) and girls (b). The results are expressed as means (95% confidence interval). Data from analysis of ANOVA and the HSD Tukey Post Hoc test. *p-value < 0.05

Another notable finding in our study is that these associations are present in girls but not in boys. The existence of sex differences in the control of eating and energy homeostasis is acknowledged[33]. A sex dichotomy in the sensitivity to central leptin and insulin has been demonstrated in rats[34]. In our study, significantly higher leptin levels were observed in prepubertal girls than in prepubertal boys. This different leptin concentration, as well as a different leptin sensitivity, in girls than in boys may contribute to explain the different findings by sex in our study. Further studies are needed to investigate the gender differences in leptin levels in prepubertal children.

With regard to the study's limitations, it is necessary to remember the inherent limitations of assessing nutrient intake through a food frequency questionnaire. The lack of body composition data in our cohort appears as another limitation of our study. Future research analyzing the findings described here in populations of other groups of age would be worthy.

In summary, the results of the present study describe that the association between leptin concentrations and dietary intake is evident only after controlling by insulin levels in 6- to 8-year-old girls in whom leptin levels are significantly higher than in boys. These facts should be taken under consideration to understand the actions of leptin regulating energy homeostasis.

## References


[1] Woods SC. The Control of Food Intake: Behavioral versus Molecular Perspectives. Cell Metab 2009;9:489–98. https://doi.org/10.1016/j.cmet.2009.04.007.

[2] Begg DP, Woods SC. The endocrinology of food intake. Nat Rev Endocrinol 2013;9:584–97. https://doi.org/10.1038/nrendo.2013.136.

[3] Varela L, Horvath TL. Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis. EMBO Rep 2012;13:1079–86. https://doi.org/10.1038/embor.2012.174.

[4] Brown LM, Clegg DJ, Benoit SC, Woods SC. Intraventricular insulin and leptin reduce food intake and body weight in C57BL/6J mice. Physiol Behav 2006;89:687–91. https://doi.org/10.1016/j.physbeh.2006.08.008.

[5] Baskin DG, Wilcox BJ, Figlewicz DP, Dorsa DM. Insulin and insulin-like growth factors in the CNS. Trends Neurosci 1988;11:107–11. https://doi.org/10.1016/0166-2236(88)90155-5.

[6] Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature 2006;443:289–95. https://doi.org/10.1038/nature05026.

[7] Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. Science (80- ) 2000;289:2122–5. https://doi.org/10.1126/science.289.5487.2122.

[8] Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci 2002;5:566–72. https://doi.org/10.1038/nn0602-861.

[9] Loh K, Zhang L, Brandon A, Wang Q, Begg D, Qi Y, et al. Insulin controls food intake and energy balance via NPY neurons. Mol Metab 2017;6:574–84. https://doi.org/10.1016/j.molmet.2017.03.013.

[10] Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet 1998;18:213–215. https://doi.org/10.1038/ng0398-213.

[11] Sadaf Farooqi I, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. Science (80- ) 2007;317:1355. https://doi.org/10.1126/science.1144599.

[12] Air EL, Benoit SC, Clegg DJ, Seeley RJ, Woods SC. Insulin and Leptin Combine Additively to Reduce Food Intake and Body Weight in Rats. Endocrinology 2002;143:2449–52. https://doi.org/10.1210/endo.143.6.8948.

[13] Kleinridders A, Lauritzen HPMM, Ussar S, Christensen JH, Mori MA, Bross P, et al. Leptin regulation of Hsp60 impacts hypothalamic insulin signaling. J Clin Invest 2013;123:4667–80. https://doi.org/10.1172/JCI67615.

[14] Thon M, Hosoi T, Ozawa K. Possible integrative actions of leptin and insulin signaling in the hypothalamus targeting energy homeostasis. Front Endocrinol (Lausanne) 2016;7:138. https://doi.org/10.3389/fendo.2016.00138.

[15] Ostlund RE, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J Clin Endocrinol Metab 1996;81:3909–13. https://doi.org/10.1210/jcem.81.11.8923837.

[16] Larsson H, Elmståhl S, Berglund G, Ahrén B. Evidence for Leptin Regulation of Food Intake in Humans. J Clin Endocrinol Metab 1998;83:4382–5. https://doi.org/10.1210/jcem.83.12.5342.

[17] Miller GD, Frost R, Olive J. Relation of plasma leptin concentrations to sex, body fat, dietary intake, and peak oxygen uptake in young adult women and men. Nutrition 2001;17:105–11. https://doi.org/10.1016/S0899-9007(00)00511-6.

[18] Yannakoulia M, Yiannakouris N, Blüher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. J Clin Endocrinol Metab 2003;88:1730–6. https://doi.org/10.1210/jc.2002-021604.

[19] Magni P, Liuzzi A, Ruscica M, Dozio E, Ferrario S, Bussi I, et al. Free and bound plasma leptin in normal weight and obese men and women: Relationship with body composition, resting energy expenditure, insulin-sensitivity, lipid profile and macronutrient preference. Clin Endocrinol (Oxf) 2005;62:189–96. https://doi.org/10.1111/j.1365-2265.2005.02195.x.

[20] Murakami K, Sasaki S, Takahashi Y, Uenishi K, Yamasaki M, Hayabuchi H, et al. Nutrient and food intake in relation to serum leptin concentration among young Japanese women. Nutrition 2007;23:461–8. https://doi.org/10.1016/j.nut.2007.04.006.

[21] Nakamura Y, Ueshima H, Okuda N, Murakami Y, Miura K, Kita Y, et al. Serum leptin and total dietary energy intake: The INTERLIPID Study. Eur J Nutr 2013;52:1641–8. https://doi.org/10.1007/s00394-012-0469-3.

[22] Miller R, Tanofsky-Kraff M, Shomaker LB, Field SE, Hannallah L, Reina SA, et al. Serum leptin and loss of control eating in children and adolescents. Int J Obes 2014;38:397–403. https://doi.org/10.1038/ijo.2013.126.

[23] Tayyem RF, Hijjawi NS, Bawadi HA, Nimer NA, Agraib LM, Allehdan SS, et al. Exploration of micro- and macronutrient consumption and leverage of interaction with adipokines among Jordanian adults. Clin Pract 2018;15:410–423. https://doi.org/10.4172/clinical-practice.1000388.

[24] Hopkins M, Finlayson G, Duarte C, Gibbons C, Johnstone AM, Whybrow S, et al. Biological and psychological mediators of the relationships between fat mass, fat-free mass and energy intake. Int J Obes 2019;43:233–42. https://doi.org/10.1038/s41366-018-0059-4.

[25] Rodríguez-Artalejo F, Garcés C, Gorgojo L, López García E, Martín-Moreno JM, Benavente M, et al. Dietary patterns among children aged 6-7 y in four Spanish cities with widely differing cardiovascular mortality. Eur J Clin Nutr 2002;56:141–8. https://doi.org/10.1038/sj.ejcn.1601296.

[26] Martin-moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-rodriguez JC, Salvini S, et al. Development and validation of a food frequency questionnaire in Spain. Int J Epidemiol 1993;22:512–9. https://doi.org/10.1093/ije/22.3.512.

[27] Navarro P, de Dios O, Jois A, Gavela-Pérez T, Gorgojo L, Martín-Moreno JM, et al. Vegetable and fruit intakes are associated with hs-CRP levels in pre-pubertal girls. Nutrients 2017;9:224. https://doi.org/10.3390/nu9030224.

[28] Woods SC, Seeley RJ. Insulin as an adiposity signal. Int J Obes 2001;25:S35–8. https://doi.org/10.1038/sj.ijo.0801909.

[29] Hillebrand JJG, Geary N. Do leptin and insulin signal adiposity? *Forum Nutr.* Basel, Karger, **2010**, 63, 111–122. https://doi.org/10.1159/000264399.

[30] Niswender KD, Schwartz MW. Insulin and leptin revisited: Adiposity signals with overlapping physiological and intracellular signaling capabilities. Front Neuroendocrinol 2003;24:1–10. https://doi.org/10.1016/S0091-3022(02)00105-X.

[31] Kolaczynski JW, Nyce MR, Considine R V., Boden G, Nolan JJ, Henry R, et al. Acute and chronic effect of insulin on leptin production in humans: Studies in vivo and in vitro. Diabetes 1996;45:699–701. https://doi.org/10.2337/diab.45.5.699.

[32] Nazarians-Armavil A, Menchella JA, Belsham DD. Cellular insulin resistance disrupts leptin-mediated control of neuronal signaling and transcription. Mol Endocrinol 2013;27:990–1003. https://doi.org/10.1210/me.2012-1338.

[33] Asarian L, Geary N. Sex differences in the physiology of eating. Am J Physiol - Regul Integr Comp Physiol 2013;305:R1215–R1267. https://doi.org/10.1152/ajpregu.00446.2012.

[34] Clegg DJ, Riedy CA, Smith KAB, Benoit SC, Woods SC. Differential sensitivity to central leptin and insulin in male and female rats. Diabetes 2003;52:682–7. https://doi.org/10.2337/diabetes.52.3.682.

## OBJETIVO 1.2. INVESTIGAR EL PAPEL QUE DESEMPEÑAN OTROS PÉPTIDOS DE SECRECIÓN CENTRAL Y PERIFÉRICA EN LA HOMEOSTASIS DE LA ENERGÍA EN AMBAS COHORTES DEL ESTUDIO 4P (NIÑOS DE 6 A 8 AÑOS Y ADOLESCENTES DE 12 A 16 AÑOS).

### OBJETIVO 1.2.1. EVALUAR EN UN ESTUDIO CASO-CONTROL LA POSIBLE ASOCIACIÓN ENTRE LOS NIVELES PLASMÁTICOS DE NESFATINA-1 Y LA PRESENCIA DE OBESIDAD.

Referencia:

**de Dios O**, Herrero L, Gavela-Pérez T, Soriano-Guillén L, Garcés C. Sex-specific association of plasma nesfatin-1 concentrations with obesity in children. Pediatr Obes 2019; 14. doi:10.1111/ijpo.12567.

#### RESUMEN

Antecedentes: La nesfatina-1 es un péptido que se ha asociado con la ingesta de alimentos y la termogénesis, con hallazgos discordantes en humanos y escasos estudios en niños hasta la fecha.

Objetivos: El objetivo de este estudio fue analizar la relación de la obesidad con los niveles de nesfatina- 1 en dos cohortes de niños.

Métodos: Se analizaron las concentraciones plasmáticas de nesfatina-1 en niños de 6 a 9 años (primera cohorte) (n = 140) y en niños de 12 a 16 años (segunda cohorte) (n = 96), incluidos niños con obesidad, pareados por edad y sexo con sus controles. Se evaluaron las medidas antropométricas. El colesterol y los triglicéridos se determinaron enzimáticamente, las concentraciones de insulina se midieron mediante radioinmunoensayo con un kit comercial y las concentraciones de nesfatina- 1, leptina y proteína C-reactiva de alta sensibilidad (as-PCR) se determinaron mediante kits de ELISA comerciales.

Resultados: Las concentraciones de nesfatina- 1 fueron significativamente más bajas en las niñas con obesidad tanto del primer (p = 0,001) como del segundo corte (p = 0,009) que en sus controles normopeso, sin mostrar diferencias significativas en los niños. Nesfatina-1 mostró una correlación negativa significativa (p <0,010) con el peso y el IMC en las niñas, pero no en los niños. En las niñas aparece una correlación positiva significativa de los niveles de nesfatina-1 con los niveles de insulina, HOMA y leptina después de ajustar por edad e IMC. En los niños/as del segundo corte se observó una correlación positiva significativa (p = 0,003) entre nesfatina- 1 y masa grasa.

Conclusiones: Nuestro estudio muestra concentraciones más bajas de nesfatina-1 relacionadas con la obesidad en las niñas, pero no en los niños en ambas cohortes de edad. La existencia de una asociación específica por sexo entre las concentraciones de nesfatina-1 y la presencia de obesidad destaca la importancia de analizar la relación de nesfatina-1 con la obesidad teniendo en cuenta el sexo.

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ORIGINAL RESEARCH

WILEY pediatricobesity

# Sex-specific association of plasma nesfatin-1 concentrations with obesity in children

Olaya de Dios[1] | Leticia Herrero[1] | Teresa Gavela-Pérez[2] | Leandro Soriano-Guillén[2] | Carmen Garcés[1]

[1] Lipid Research Laboratory, IIS-Fundación Jiménez Díaz, Madrid, Spain

[2] Department of Pediatrics, IIS-Fundación Jiménez Díaz, Madrid, Spain

**Correspondence**
Carmen Garcés, Lipid Research Laboratory, IIS-Fundación Jiménez Díaz, Avda. Reyes Católicos, 2. 28040 Madrid, Spain.
Email: cgarces@fjd.es

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## Summary

**Background:** Nesfatin-1, an anorexigenic peptide, has been associated with food intake and thermogenesis, with discordant findings in humans and scarce studies in children to date.

**Objectives:** The aim of this study was to analyze the relationship of obesity with nesfatin-1 levels in two cohorts of children.

**Methods:** Plasma nesfatin-1 concentrations were analyzed in 6- to 9-year-olds ($n$ = 140) and 12- to 16-year-old children ($n$ = 96), including children with obesity and their sex- and age-matched normal-weight counterparts. Anthropometric measurements were assessed. Cholesterol and triglycerides were determined enzymatically, insulin concentrations were measured by radioimmunoassay using a commercial kit and nesfatin-1, leptin and hs-CRP concentrations were determined using commercial ELISA kits.

**Results:** Nesfatin-1 concentrations were significantly lower in younger ($P$ = .001) and older ($P$ = .009) girls with obesity than in their normal-weight counterparts, without showing significant differences in boys. Nesfatin-1 showed a negative significant ($P$ < .010) correlation with weight and BMI in girls but not in boys. A significant positive correlation of nesfatin-1 levels with insulin, HOMA, and leptin levels appears in girls after adjusting by age and BMI. A significant positive correlation ($P$ = .003) was observed between nesfatin-1 and fat mass in older children.

**Conclusions:** Our study shows lower concentrations of nesfatin-1 related to obesity in girls but not in boys at two different ages. The existence of a sex-specific association between nesfatin-1 concentrations and presence of obesity highlights the need of an analysis by gender of the relationship of nesfatin-1 with obesity.

KEYWORDS

body mass index, children, gender, nesfatin-1 levels, obesity

## 1 | INTRODUCTION

Obesity results from an imbalance between energy intake and expenditure. Food intake is regulated by a complex appetite control system, in which brain hypothalamus-secreted molecules play an important role. Nesfatin-1 is an anorexigenic peptide encoded in the precursor nucleobindin-2 [NUCB2] that was found to express in the appetite-control hypothalamic nuclei in rats,[1] and afterward, it was implicated in the regulation of food intake in rodents.[2] Thus, this novel neuropeptide appears to play an important role in hypothalamic pathways

  

regulating food intake and subsequently body weight, besides being implicated in other important brain functions such as reproduction, sleep, cognition, and anxiety- or stress-related responses.[3,4]

A substantial number of studies in animal models have linked nesfatin-1 to food intake and satiety.[2] In these studies, the anorexigenic effect of nesftain-1 has been clearly observed after its central injection, giving rise to the assumption of a predominantly central mode of anorexigenic action. For example, several studies in rats[5-7] and mice[8,9] shown that brain ventricular injection of NUCB2 reduces food intake in a dose-dependent manner, ending in a body weight reduction.[10] On the other hand, injection of an antibody neutralizing nesfatin-1 stimulates appetite.[1]

However, being secreted in the brain by neurons present in energy-regulating areas, nesfatin-1 is also expressed in peripheral tissues, such as adipose tissue, heart, gonads, pancreas, stomach, or liver,[11,12] and its role as a peripheral hormone is under study.[13] It has been reported that peripheral administration of nesfatin-1 inhibits food intake in mice in a leptin-independent mechanism.[14] Similarly, it has been shown that chronic subcutaneous infusion of nesfatin-1 reduces food intake and modulates body energy homeostasis in rats.[15]

In humans, different analyses of the association of nesfatin-1 with obesity have yielded divergent results. While some studies have reported negative correlations between nesfatin-1 levels and obesity or body mass index (BMI) in adults[16,17] and children,[18,19] some have failed to find any correlation[20-22] and others have reported a positive relationship between nesfatin-1 concentrations and weight status in adults[23,24] and childhood.[25,26]

Therefore, the aim of our study was to try to clarify the relationship between nesfatin-1 and obesity, analyzing plasma nesfatin-1 concentrations in 6- to 9-year-old and 12- to 16-year-old children with obesity and in their sex and age-matched normal-weight counterparts.

## 2 | METHODS

### 2.1 | Subjects

Our population-based samples comprise a homogeneous population of 6- to 9-year-old children (70 with obesity and 70 sex-paired children with normal weight), and another cohort of children aged 12 to 16 years (48 with obesity and 48 sex-paired children with normal weight).

Subjects in our study were selected from participants in a cross-sectional study designed to examine cardiovascular risk factors in Spain. In this cross-sectional study, data was gathered from representative samples of children in four areas over the periods 1998–1999 (6- to 9-year-old children) and 2006–2008 (12- to 16-year-old children).[27,28] The sample included in the present study comprises children identified as having obesity in both groups of age in our cross-sectional study, as well as a similar number of age and sex-matched children selected as controls. To rule out the possibility of the values of any of the variables of interest being altered, all children reported by parents to be suffering from chronic diseases, including precocious and delayed puberty, were excluded.

Children in our cross-sectional study were selected by means of random cluster-sampling in schools, and stratified by sex and type of school (ie, public versus private). Sampling was carried out in two stages: first, schools were selected from lists made available by the Regional Educational Authorities; and second, classrooms and pupils were selected.

Written consent was obtained from all parents before their children were included in the study. The study protocol was approved by the Ethics Committee of Clinical Investigation of the Fundación Jiménez Díaz. The investigation fulfils the principles contained in the Declaration of Helsinki and subsequent reviews, as well as the prevailing Spanish legislation on clinical research in human subjects.

### 2.2 | Anthropometric data

Measurements (weight and height) were taken with children wearing light clothing and barefoot. Height was measured to the millimeter using a portable stadiometer, and weight was recorded to the nearest 0.1 kg using a standard electronic digital scale. BMI (weight in kilograms/height in meters squared) was calculated from these measurements. *Z*-score BMI was also calculated according to Spanish reference data. Children were classified as having obesity if their BMI exceeded the age- and sex-specific cut-off points established for children by Cole et al.[29]

### 2.3 | Biochemical data

Blood samples were obtained early in the morning after a 12-hour fasting period by venipuncture into Vacutainer tubes that contained EDTA-$Na^2$ as an anticoagulant and were kept cold from the time of collection. Blood samples were centrifuged (1500*g* at 4°C for 25 min), and plasma was separated in aliquots, which were immediately stored at −80°C for further analysis. Cholesterol and triglycerides (TG) were determined enzymatically with a Technicon RA-1000 Autoanalyzer (Technicon, Dublin, Ireland). The inter-assay coefficients of variation were 2.1% for cholesterol and 3.4% for triglycerides. Insulin concentrations were measured by radioimmunoassay using a commercial kit (BI-Insulin IRMA; Bio-Rad, France). Insulin resistance was estimated using the HOMA score [fating insulin (μU/ml) × fasting glucose (mmol/L)/22.5]. CRP levels were measured using an hs-CRP ELISA kit (SK00080-02; Aviscera Bio- science, Santa Clara, California) with intra- and inter-assay coefficients of variation of 6% and 12%, respectively. Leptin concentrations were determined by ELISA using a commercially available kit (Leptin EIA-2395, DRG Instruments GmbH, Marburg, Germany), with intra- and inter-assay coefficients of variation of 6.9% and 8.6%, respectively. Nesfatin-1 concentration was measured using a human nesfatin-1 ELISA kit (RD191227200R, BioVendor, Brno, Czech Republic), intra- and inter-assay coefficient of variation were 1.8% and 3.2%, respectively.

### 2.4 | Statistical analysis

Descriptive statistics, including means and 95% confidence intervals (CI), were performed for all variables by age category and gender. Each variable was examined for normal distribution using the Kolmogorov–Smirnov test. Given their skewed distributions, concentrations of weight, BMI, triglycerides, insulin, HOMA, leptin, and hs-CRP were log-transformed before statistical comparison. Inter-group comparisons of means were performed using the Student's *t* test. All comparisons were two-sided at a 0.05 significance level. Partial correlations, adjusting by age and BMI, were tested using the log-transformed data as indicated. Statistical analysis was performed using the SPSS software package, version 21.0, and GraphPad Prism statistical software, version 6.

## 3 | RESULTS

The study comprises a total of 236 Spanish children of two groups of age: 6- to 9-year-olds (n = 140) and 12- to 16-year-olds (n = 96). Age, anthropometric, and biochemical parameters in children of both groups of age by sex and weight category are shown in Table 1. The average ages of the children were 7.2 and 14.3 years in younger and older children, respectively, with no significant differences between sexes or weight category. Boys and girls with obesity had significantly higher insulin levels and HOMA than their normal weight counterparts in both groups of age (Table 1). No differences in plasma glucose levels were found. Leptin levels were significantly higher ($P < .001$) in girls than boys and in children with obesity than in children with normal weight ($P < .001$) in both groups of age.

Plasma nesfatin-1 levels in younger and older children according to weight category and gender are shown in Figure 1. Nesfatin-1 levels were significantly higher in girls with normal weight than in girls with obesity in both groups of age: 5.7 (3.5-8.0) and 2.3 (1.1-3.4) ng mL$^{-1}$, respectively, ($P < .005$) in younger girls and 5.5 (2.0-9.0) and 2.5 (0.4-4.7) ng mL$^{-1}$, respectively, ($P < .05$) in older girls. No significant differences were found in boys (Figure 1).

To further analyze the relationship between nesfatin-1 and anthropometric and biochemical variables, a partial correlation analysis controlled by age was performed (Table 2). The results show a significant negative correlation between nesfatin-1 levels and weight ($r = -.241$, $P = .007$) and BMI ($r = -.249$, $P = .005$) in girls, while no correlation was observed in boys. A significant negative correlation between nesfatin-1 levels and hs-CRP ($r = -.246$, $P = .024$) was also observed in girls. There was no correlation between nesfatin-1 levels and any other of the analyzed variables in any gender (Table 2). When performing partial correlation analysis controlled by age and BMI between nesfatin-1 and biochemical variables (Table 3), a significant positive correlation of nesfatin-1 levels with insulin, HOMA, and leptin levels appears in girls. No correlations were observed between nesfatin-1 and cholesterol, TG, or hs-CRP concentrations.

Finally, we examined the correlation between nesfatin-1 levels and fat mass in 22 and 18 older boys and girls, respectively, of whom 20 were children with obesity and 20 with normal weight. According to partial correlation analysis controlled by BMI, there was a significant positive correlation between nesfatin-1 levels and fat mass ($r = .481$, $P = .002$).

## 4 | DISCUSSION

In our study, analyzing the association between nesfatin-1 levels and presence of obesity in children, we described a significantly lower concentration of nesfatin-1 in girls with obesity than in their normal-weight counterparts at two different ages: 6- to 9-year-old and 12- to 16-year-old girls. Additionally, we found significant negative correlations of nesfatin-1 levels with weight and BMI in girls of both age groups. However, we failed to find these associations in boys.

Studies analyzing these issues in adults and children have yielded contradictory results.[16-25] Although nesfatin-1 levels have been negatively correlated with BMI in healthy normal-weight males,[16] most of the studies in adults analyzing the relationship between nesfatin-1 concentrations and BMI or presence of obesity have been performed in populations with different pathologies, such as patients with morbid obesity with type 2 diabetes mellitus[30,31] or subjects with anorexia.[32] The divergent results found in these studies could be related to factors associated with these pathologies.

To our knowledge, only two studies have been performed comparing nesfatin-1 concentrations in children with normal weight and children with obesity, and these studies have shown discordant results. Abaci et al[18] reported lower nesfatin-1 levels in children with obesity than in healthy children, while Anwar et al[26] found the opposite results analyzing children and young adolescents. In addition, different results were also observed in two studies in children with chronic malnutrition.[19,25] None of the studies in children have analyzed the association of nesfatin-1 concentrations with obesity or anthropometric parameters by gender.

The reason behind the sex-specific association of nesfatin-1 with the presence of obesity we described here is unclear. However, the central nervous system actions of nesfatin-1 reducing food intake is widely accepted,[1,4] and a sex-specific central regulation of NUCB2/nesfatin-1 has been reported by Bloem et al[33] investigating the brain expression of NUCB2/nesfatin-1. Besides, it has been described that NUCB2/nesfatin-1 is involved in the regulation of mood and behavioral response to stress in a sex-specific way. Hofmann et al[20] reported that circulating NUCB2/nesfatin-1 was related to anxiety, perceived stress, and depressiveness in women with obesity, but not in men with obesity, pointing towards a sex-specific regulation.[20] On the basis of these findings, we could speculate that a different central regulation of nesfatin-1 depending on sex could justify that the correlation between nesfatin-1 and weight and BMI is observed only in girls.

On the other hand, in our older children, we described a significant correlation between nesfatin-1 and body fat. As discussed above, the central action of nesfatin-1 as a physiological anorexigenic peptide is well established, while the action of peripheral nesfatin-1 is less clear.[4]

**TABLE 1** Characteristics of the prepubertal and pubertal children with normal weight and with obesity

| | 6- to 9-year-olds | | | | 12- to 16-year-olds | | | |
|---|---|---|---|---|---|---|---|---|
| | Boys | | Girls | | Boys | | Girls | |
| | NW (n = 29) | OB (n = 29) | NW (n = 41) | OB (n = 41) | NW (n = 27) | OB (n = 27) | NW (n = 21) | OB (n = 21) |
| Age, years | 7.2 (7.0-7.5) | 7.3 (7.1-7.5) | 7.3 (7.0-7.5) | 7.2 (7.0-7.4) | 14.4 (14.0-14.8) | 14.5 (14.1-14.9) | 14.1 (13.4-14.7) | 14.1 (13.5-14.8) |
| Weight, kg | 23.4 (22.3-24.5) | 35.5 (36.8-40.2) | 22.9 (21.8-23.9) | 36.7 (35.1-38.3) | 48.7 (45.7-51.8) | 92.5 (87.6-97.4) | 47.1 (44.1-50.0) | 82.8 (77.2-88.4) |
| BMI, kg/m$^2$ | 15.0 (14.6-15.4) | 22.6 (22.0-23.2) | 14.8 (14.4-15.1) | 22.3 (21.8-22.8) | 18.0 (17.5-18.6) | 31.8 (30.5-33.2) | 18.0 (17.4-18.6) | 31.6 (30.0-33.2) |
| BMI z-score | −0.8 (−0.9-−0.7) | 2.3 (2.1-2.5) | −0.8 (−1.0-−0.7) | 2.1 (1.9-2.3) | −0.9 (−1.1-−0.8) | 2.7 (2.2-3.2) | −0.8 (−1.0-−0.7) | 2.9 (2.4-3.5) |
| Glucose, mg/dL | 94.82 (91.8-97.8) | 95.2 (92.2-98.1) | 86.8 (83.8-89.7) | 91.3 (87.4-95.1) | 91.6 (88.1-95.2) | 94.5 (89.2-99.8) | 87.6 (80.7-94.5) | 91.7 (89.1-94.3) |
| Insulin, uUI/mL | 2.4 (1.9-2.8) | 5.6 (4.5-6.6) | 3.0 (1.9-4.1) | 6.2 (4.9-7.4) | 6.7 (5.6-7.9) | 12.8 (9.7-16.0) | 8.3 (4.9-11.6) | 15.5 (11.6-19.4) |
| HOMA | 0.5 (0.4-0.6) | 1.3 (1.1-1.6) | 0.6 (0.4-0.9) | 1.4 (1.1-1.7) | 1.5 (1.3-1.7) | 3.0 (2.2-3.8) | 1.6 (1.2-2.0) | 3.5 (2.6-4.3) |
| Total cholesterol, mg/dL | 186.9 (177.5-196.3) | 179.3 (170.0-188.5) | 181.0 (171.2-190.7) | 179.0 (170.3-187.7) | 168.0 (155.8-180.1) | 164.4 (149.9-178.9) | 155.5 (142.0-169.0) | 174.4 (163.1-185.7) |
| Triglycerides, mg/dL | 77.1 (69.6-84.6) | 90.4 (76.8-104.0) | 69.1 (63.6-74.5) | 88.8 (79.8-97.7) | 72.2 (56.6-87.9) | 97.9 (82.6-113.1) | 66.1 (56.8-75.4) | 84.0 (68.5-99.6) |
| Hs-CRP, mg/L | 0.2 (0.1-0.3) | 1.3 (0.8-1.8) | 0.7 (−0.1-1.4) | 1.3 (0.9-1.8) | 0.4 (0.2-0.7) | 1.9 (1.2-2.6) | 0.6 (0.2-1.1) | 1.5 (1.1-2.0) |
| Leptin, ng/mL | 1.4 (1.0-1.9) | 17.7 (12.6-22.8) | 5.8 (3.3-8.3) | 27.2 (22.8-36.1) | 1.4 (0.6-2.2) | 21.6 (17.3-25.8) | 9.4 (6.9-12.0) | 36.5 (32.1-41.0) |

*Note*. Data are expressed as mean (95% confidence interval).

Abbreviation: BMI, body mass index; NW, normal-weight children; OB, children with obesity.

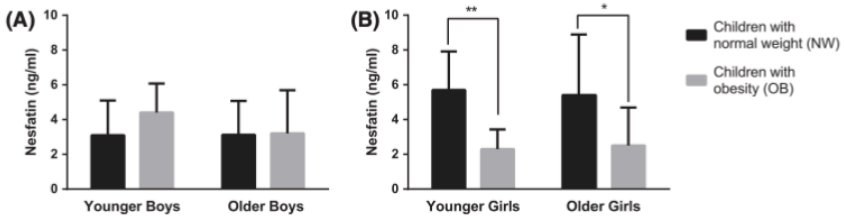


**FIGURE 1** Nesfatin-1 levels (ng/mL) by weight category in younger and older boys (a) and girls (b). The results are expressed as means (95% confidence interval). *P* values by *t* test analysis. $^{**}P$ value < .005, $^{*}P$ value < .05. NW, children with normal weight; OB, children with obesity.

**TABLE 2** Partial correlation, controlled by age, between nesfatin-1 and anthropometric measures and biochemical variables in boys and girls

| | Boys, n = 112 | | Girls, n = 124 | |
|---|---|---|---|---|
| | *r* | *P* | *r* | *P* |
| Weight, kg[a] | −0.100 | *0.296* | −0.241 | 0.007 |
| BMI,[a] kg/m$^2$ | −0.072 | *0.456* | −0.249 | 0.005 |
| Glucose, mg/dL | 0.009 | 0.925 | −0.002 | 0.978 |
| Insulin, uUI/mL[a] | 0.121 | 0.220 | 0.060 | 0.521 |
| HOMA[a] | 0.116 | 0.243 | 0.055 | 0.556 |
| Total cholesterol | −0.093 | 0.334 | −0.069 | 0.445 |
| Triglycerides,[a] mg/dL | 0.002 | 0.983 | 0.040 | 0.659 |
| Hs-CRP, mg/L[a] | −0.082 | 0.459 | −0.246 | 0.024 |
| Leptin, ng/mL[a] | −0.002 | 0.987 | −0.050 | 0.634 |

Abbreviations: BMI, body mass index; *P*, *P* value; *r*, correlation.

[a]log transformed.

**TABLE 3** Partial correlation, controlled by age and BMI, between nesfatin-1 and biochemical variables in boys and girls

| | Boys n = 112 | | Girls n = 124 | |
|---|---|---|---|---|
| | *r* | *P* | *r* | *P* |
| Glucose, mg/dL | 0.021 | 0.831 | 0.047 | 0.607 |
| Insulin, uUI/mL[a] | 0.192 | 0.051 | 0.210 | 0.022 |
| HOMA[a] | 0.185 | 0.061 | 0.212 | 0.021 |
| Total cholesterol | −0.097 | 0.316 | −0.066 | 0.607 |
| Triglycerides[a] mg/dL | 0.021 | 0.826 | 0.044 | 0.633 |
| Hs-CRP, mg/L[a] | −0.095 | 0.395 | −0.167 | 0.132 |
| Leptin, ng/mL[a] | 0.116 | 0.326 | 0.261 | 0.011 |

Abbreviations: BMI, body mass index; *P*, *P* value; *r*, correlation

[a]log transformed

Consistent with our results, some studies in adults have described positive associations of plasma nesfatin-1 with fat mass.[24,34,35] However, the study of Tan et al[24] in adults reported a positive correlation between nesfatin-1 and BMI and fat mass, while, at the same time, they found a significant negative association between the cerebrospinal fluid/plasma nesfatin-1/NUCB-2 ratio and these parameters. They propose that a substantial amount of nesfatin-1 may originate from central neurons,[24] which has an important anorexigenic action. These evidences allow us to hypothesize an interpretation of our results that show a significant positive correlation of nesfatin-1 with fat mass and with leptin after adjusting by BMI, but show lower nefastin-1 in girls with obesity. We could think that beyond the peripheral regulation of nesfatin-1 related to adipose tissue, which would justify the positive correlations described in our study, an important secretion of nesfatin-1 derives from central control which, as discussed above, has an anorexigenic action and shows a sex-specific pattern.

Also, in our study, a positive correlation of nesfatin-1 with insulin levels and HOMA is present in girls only when adjusting by BMI. Positive correlations between nesfatin-1 and insulin have been described in some studies in adults and children,[24,26,36] but again in the study of Tan et al,[24] although a positive correlation between nesfatin-1 and insulin and HOMA is observed, a significant negative correlation between the cerebrospinal fluid/plasma nesfatin-1/NUCB-2 ratio and insulin and HOMA is reported. In our study, the correlation between nesfatin-1 and insulin is not evident unless the analysis is adjusted by BMI, supporting again the fact of a large amount of nesfatin-1 with a central source, and a secondary contribution of peripheral tissues to plasma nesfatin-1 levels.

Several limitations of our study should be taken under consideration. The absence of information on pubertal stage assessment in our children, which prevents us from classifying our children in prepubertal and pubertal; the lack of information on fat mass in an important number of children in our study, which makes a deeper analysis of the relationship between fat mass and nesfatin-1 difficult; the fact that significant potential changes in the environment may be affecting; and finally the fact that interpersonal variability could be related to factors such as diet and physical activity that are not analyzed in our study. Further studies specifically designed to evaluate the relationship between diet and nesfatin-1 should be performed.

In summary, our study is the first to analyse the association between nesfatin-1 and obesity by gender in children and describe that this association is sex-specific, as significant lower nesfatin-1 concentrations have been found in girls but not in boys with obesity. This difference could be related to the sex-specific secretion of nesfatin-1 by the central nervous system. Our findings emphasize the need to analyse by sex the role of nesfatin-1 in relationship to obesity.

## ACKNOWLEDGEMENTS

The article is dedicated to the late Prof. Manuel de Oya as the warmest homage to his memory. Prof. de Oya designed the Four Province Study and the ideas reflected in our work can be traced back to his.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

Olaya de Dios and Leticia Herrero carried out laboratory work, participated substantially in data analysis and reviewed the manuscript; Teresa Gavela-Pérez and Leandro Soriano-Guillen reviewed the manuscript and made contributions to the interpretation of data; Carmen Garcés designed and conducted the study, analyzed the data, and wrote the manuscript.

## ORCID

*Carmen Garcés* https://orcid.org/0000-0002-1099-2061

## REFERENCES

1. Oh-I S, Shimizu H, Satoh T, et al. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature*. 2006;443(7112):709-712. https://doi.org/10.1038/nature05162
2. Dore R, Levata L, Lehnert H, Schulz C. Nesfatin-1: functions and physiology of a novel regulatory peptide. *J Endocrinol*. 2017;232(1):R45-R65. https://doi.org/10.1530/JOE-16-0361
3. Pałasz A, Krzystanek M, Worthington J, et al. Nesfatin-1, a unique regulatory neuropeptide of the brain. *Neuropeptides*. 2012;46(3):105-112. https://doi.org/10.1016/j.npep.2011.12.002
4. Stengel A, Mori M, Taché Y. The role of nesfatin-1 in the regulation of food intake and body weight: recent developments and future endeavors. *Obes Rev*. 2013;14(11):859-870. https://doi.org/10.1111/obr.12063
5. Stengel A, Goebel M, Wang L, et al. Central nesfatin-1 reduces dark-phase food intake and gastric emptying in rats: differential role of corticotropin-releasing factor $_2$ receptor. *Endocrinology*. 2009;150(11):4911-4919. https://doi.org/10.1210/en.2009-0578
6. Yosten GL, Samson WK. The anorexigenic and hypertensive effects of nesfatin-1 are reversed by pretreatment with an oxytocin receptor antagonist. *Am J Physiol Integr Comp Physiol*. 2010;298(6):R1642-R1647. https://doi.org/10.1152/ajpregu.00804.2009
7. Könczöl K, Pintér O, Ferenczi S, et al. Nesfatin-1 exerts long-term effect on food intake and body temperature. *Int J Obes (Lond)*. 2012;36(12):1514-1521. https://doi.org/10.1038/ijo.2012.2
8. Atsuchi K, Asakawa A, Ushikai M, et al. Centrally administered nesfatin-1 inhibits feeding behaviour and gastroduodenal motility in mice. *Neuroreport*. 2010;21(15):1. https://doi.org/10.1097/WNR.0b013e32833f7b96
9. Goebel M, Stengel A, Wang L, Taché Y. Central nesfatin-1 reduces the nocturnal food intake in mice by reducing meal size and increasing inter-meal intervals. *Peptides*. 2011;32(1):36-43. https://doi.org/10.1016/j.peptides.2010.09.027
10. Yuan JH, Chen X, Dong J, et al. Nesfatin-1 in the lateral parabrachial nucleus inhibits food intake, modulates excitability of glucosensing neurons, and enhances UCP1 expression in brown adipose tissue. *Front Physiol*. 2017;8(APR):235. https://doi.org/10.3389/fphys.2017.00235
11. Stengel A, Taché Y. Minireview: nesfatin-1—an emerging new player in the brain-gut, endocrine, and metabolic Axis. *Endocrinology*. 2011;152(11):4033-4038. https://doi.org/10.1210/en.2011-1500
12. Schalla MA, Stengel A. Current understanding of the role of nesfatin-1. *J Endocr Soc*. 2018;2(10):1188-1206. https://doi.org/10.1210/js.2018-00246
13. Prinz P, Stengel A. Nesfatin-1: current status as a peripheral hormone and future prospects. *Curr Opin Pharmacol*. 2016;31:19-24. https://doi.org/10.1016/j.coph.2016.08.011
14. Shimizu H, Oh-I S, Hashimoto K, et al. Peripheral administration of nesfatin-1 reduces food intake in mice: the leptin-independent mechanism. *Endocrinology*. 2009;150(2):662-671. https://doi.org/10.1210/en.2008-0598
15. Mortazavi S, Gonzalez R, Ceddia R, Unniappan S. Long-term infusion of nesfatin-1 causes a sustained regulation of whole-body energy homeostasis of male Fischer 344 rats. *Front Cell Dev Biol*. 2015;3:22. https://doi.org/10.3389/fcell.2015.00022
16. Tsuchiya T, Shimizu H, Yamada M, et al. Fasting concentrations of nesfatin-1 are negatively correlated with body mass index in non-obese males. *Clin Endocrinol (Oxf)*. 2010;73(4):484-490. https://doi.org/10.1111/j.1365-2265.2010.03835.x
17. Psilopanagioti A, Nikou S, Papadaki H. Nucleobindin-2/nesfatin-1 in the human hypothalamus is reduced in obese subjects and colocalizes with oxytocin, vasopressin, melanin-concentrating hormone, and cocaine- and amphetamine-regulated transcript. *Neuroendocrinology* January. 2019;108(3):190-200. https://doi.org/10.1159/000496731
18. Abaci A, Catli G, Anik A, Kume T, Bober E. The relation of serum nesfatin-1 level with metabolic and clinical parameters in obese and healthy children. *Pediatr Diabetes*. 2013;14(3):189-195. https://doi.org/10.1111/pedi.12009
19. Acar S, Çatli G, Küme T, et al. Increased concentrations of serum nesfatin-1 levels in childhood with idiopathic chronic malnutrition. *Turkish J Med Sci*. 2018;48(2):378-385. https://doi.org/10.3906/sag-1705-20
20. Hofmann T, Elbelt U, Ahnis A, Rose M, Klapp BF, Stengel A. Sex-specific regulation of NUCB2/nesfatin-1: differential implication in anxiety in obese men and women. *Psychoneuroendocrinology*. 2015;60:130-137. https://doi.org/10.1016/j.psyneuen.2015.06.014
21. Hofmann T, Stengel A, Ahnis A, Bußе P, Elbelt U, Klapp BF. NUCB2/nesfatin-1 is associated with elevated scores of anxiety in female obese patients. *Psychoneuroendocrinology*. 2013;38(11):2502-2510. https://doi.org/10.1016/j.psyneuen.2013.05.013
22. Çelik F, Belviranli M, Okudan N. Circulating levels of leptin, nesfatin-1 and kisspeptin in postmenopausal obese women. *Arch Physiol Biochem*. 2016;122(4):195-199. https://doi.org/10.3109/13813455.2016.1171365
23. Ramanjaneya M, Chen J, Brown JE, et al. Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. *Endocrinology*. 2010;151(7):3169-3180. https://doi.org/10.1210/en.2009-1358
24. Tan BK, Hallschmid M, Kern W, Lehnert H, Randeva HS. Decreased cerebrospinal fluid/plasma ratio of the novel satiety molecule, nesfatin-1/NUCB-2, in obese humans: evidence of nesfatin-1/NUCB-2 resistance and implications for obesity treatment. *J Clin Endocrinol Metab*. 2011;96(4):E669-E673. https://doi.org/10.1210/jc.2010-1782

25. Kahraman FU, Vehapoğlu A, Özgen İT, Terzioğlu Ş, Cesur Y, Dündaröz R. Correlation of brain neuropeptide (Nesfatin-1 and orexin-a) concentrations with anthropometric and biochemical parameters in malnourished children. *JCRPE J Clin Res Pediatr Endocrinol*. 2015;7(3):197-202. https://doi.org/10.4274/jcrpe.1930

26. Anwar GM, Yamamah G, Ibrahim A, El-Lebedy D, Farid TM, Mahmoud R. Nesfatin-1 in childhood and adolescent obesity and its association with food intake, body composition and insulin resistance. *Regul Pept*. 2014;188:21-24. https://doi.org/10.1016/j.regpep.2013.12.001

27. Garcés C, Gutierrez-Guisado J, Benavente M, et al. Obesity in Spanish schoolchildren: relationship with lipid profile and insulin resistance. *Obes Res*. 2005;13(6):959-963. https://doi.org/10.1038/oby.2005.111

28. Schoppen S, Riestra P, García-Anguita A, et al. Leptin and adiponectin levels in pubertal children: relationship with anthropometric variables and body composition. *Clin Chem Lab Med*. 2010;48(5):707-711. https://doi.org/10.1515/CCLM.2010.142

29. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. 2000;320(7244):1240-1243. http://www.ncbi.nlm.nih.gov/pubmed/10797032 Accessed February 9, 2019

30. Lee W-J, Chen C-Y, Ser K-H, et al. Differential influences of gastric bypass and sleeve gastrectomy on plasma nesfatin-1 and obestatin levels in patients with type 2 diabetes mellitus. *Curr Pharm des*. 2013;19(32):5830-5835. https://doi.org/10.2174/13816128113198880010

31. Dogan U, Bulbuller N, Cakır T, et al. Nesfatin-1 hormone levels in morbidly obese patients after laparoscopic sleeve gastrectomy. *Eur Rev Med Pharmacol Sci*. 2016;20(6):1023-1031. http://www.ncbi.nlm.nih.gov/pubmed/27049252 Accessed October 17, 2018

32. Ogiso K, Asakawa A, Amitani H, et al. Plasma nesfatin-1 concentrations in restricting-type anorexia nervosa. *Peptides*. 2011;32(1):150-153. https://doi.org/10.1016/j.peptides.2010.10.004

33. Bloem B, Xu L, Morava É, et al. Sex-specific differences in the dynamics of cocaine- and amphetamine-regulated transcript and nesfatin-1 expressions in the midbrain of depressed suicide victims vs. controls. *Neuropharmacology*. 2012;62(1):297-303. https://doi.org/10.1016/j.neuropharm.2011.07.023

34. Mirzaei K, Hossein-Nezhad A, Keshavarz SA, et al. Association of nesfatin-1 level with body composition, dietary intake and resting metabolic rate in obese and morbid obese subjects. *Diabetes Metab Syndr Clin Res Rev*. 2015;9(4):292-298. https://doi.org/10.1016/j.dsx.2014.04.010

35. Xun C, Xiao-Min L, Li-Hong Z. Recent progress in research on the distribution and function of NUCB2/Nesfatin-1 in peripheral tissues. *Endocr J*. 2013;60(9):1021-1027.

36. Zhang Z, Li L, Yang M, Liu H, Boden G, Yang G. Increased plasma levels of nesfatin-1 in patients with newly diagnosed type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. 2012;120(2):91-95. https://doi.org/10.1055/s-0031-1286339

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## OBJETIVO 1.2. INVESTIGAR EL PAPEL QUE DESEMPEÑAN OTROS PÉPTIDOS DE SECRECIÓN CENTRAL Y PERIFÉRICA EN LA HOMEOSTASIS DE LA ENERGÍA EN AMBAS COHORTES DEL ESTUDIO 4P (NIÑOS DE 6 A 8 AÑOS Y ADOLESCENTES DE 12 A 16 AÑOS).

### OBJETIVO 1.2.2. ANALIZAR EN UN ESTUDIO CASO-CONTROL LA POSIBLE RELACIÓN ENTRE LOS NIVELES PLASMÁTICOS DE ADROPINA Y LA PRESENCIA DE OBESIDAD.

Referencia:

Herrero L, **de Dios O**, Gavela-Pérez T, Riestra P, Jois A, Soriano-Guillén L, Garcés C. Opposite Association of Adropin Concentrations with Obesity in Prepubertal Children Compared with Adolescents. Obesity 2020; 28: 1736–1741.


#### RESUMEN

Objetivo: El objetivo de este estudio fue evaluar la posible asociación entre la presencia de obesidad y los niveles plasmáticos de adropina en edad pediátrica en dos cohortes de edades diferentes.

Métodos: Se midieron las concentraciones de adropina en 71 niños prepúberes y 41 púberes con obesidad y sus controles normopeso apareados según edad y sexo. En estos niños se disponía de información sobre los niveles de insulina, el perfil lipídico y los niveles de leptina. Los niveles de adropina se midieron utilizando un kit de ensayo inmunoabsorbente ligado a enzimas comercial.

Resultados: Los niveles plasmáticos de adropina fueron significativamente más altos ($p < 0,001$) en los niños prepúberes que en los púberes. Las concentraciones de adropina fueron significativamente más altas ($p < 0,001$) en las niñas prepúberes que en los niños prepúberes, pero significativamente más bajas ($p < 0,001$) en las niñas púberes que en los niños púberes. Los niños y niñas prepúberes con obesidad presentaron niveles de adropina significativamente más altos ($p < 0,001$) en comparación a sus controles normopeso. Por el contrario, no se observaron diferencias en los niveles de adropina ni en los niños ni en las niñas púberes al comparar el grupo de obsesos con el de normopeso.

Conclusiones: Se demostró una importante disminución de los niveles de adropina en niños púberes en comparación con los prepúberes, así como una asociación diferente de adropina con obesidad según la edad. Estos hallazgos sugieren una posible relación entre los niveles de adropina y las hormonas sexuales involucradas en el desarrollo puberal.

**Original Article**
PEDIATRIC OBESITY

Obesity

# Opposite Association of Adropin Concentrations with Obesity in Prepubertal Children Compared with Adolescents

*Leticia Herrero[1], Olaya de Dios[1], Teresa Gavela-Pérez[2], Pía Riestra[3], Asha Jois[4], Leandro Soriano-Guillén[2], and Carmen Garcés [1]*

**Objective:** The aim of this study was to evaluate the association between obesity and plasma adropin levels in two cohorts of children at two different ages.

**Methods:** Adropin concentrations were measured in 71 prepubertal and 41 pubertal children with obesity and their age- and sex-matched normal weight counterparts (69 prepubertal and 42 pubertal children). Information was available in these children on insulin levels, lipid profile, and leptin levels. Adropin levels were measured by using a commercial enzyme-linked immunosorbent assay kit.

**Results:** Plasma adropin levels were significantly higher ($P<0.001$) in prepubertal than pubertal children. Adropin concentrations were significantly higher ($P<0.001$) in prepubertal girls than in prepubertal boys but significantly lower ($P<0.001$) in pubertal girls than in pubertal boys. Prepubertal boys and girls with obesity had significantly higher adropin levels ($P<0.001$) than their normal weight counterparts. In contrast, no differences in adropin levels were observed in pubertal children when comparing children with obesity and normal weight boys and girls.

**Conclusions:** An important decrease in adropin levels in pubertal children compared with prepubertal children was shown as well as a differing association of adropin with obesity depending on age. These findings suggest a possible relationship between adropin levels and centrally regulated sex hormones involved in pubertal development.

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## Study Importance

### What is already known?

- Adropin is involved in body weight regulation and glucose and lipid homeostasis in animals.
- Studies addressing the role of adropin in metabolism homeostasis in humans have provided divergent results.

### What does this study add?

- There is an age-dependent relationship of plasma adropin levels with obesity in children.
- We report an opposite direction of the association between adropin concentration and gender in pubertal and prepubertal children.
- This study suggests a possible relationship between adropin levels and centrally regulated hormones involved in pubertal development.

### How might these results change the direction of research?

- Our results should lead to further studies designed to clarify the influence of sexual hormones on the association between obesity and adropin and other energy homeostasis regulatory peptides.

## Introduction

Obesity has been predominantly associated with peptides secreted by adipose tissue, known as adipokines (1). However, in recent years, it has been found that additional proteins secreted from different organs also appear to be key factors in energy homeostasis. Adropin has emerged as one of these new peptides (2).

Secreted adropin protein is composed of 43 amino acids and is produced by proteolytic cleavage of 76 amino acid precursors (3). Although its precise functions are not known, studies in humans have suggested that adropin may be implicated in metabolic homeostasis and cardiovascular function (4). Although it has been proposed that the biological effects of adropin could be mediated by interaction with the G protein–coupled receptor

[1] Lipid Research Laboratory, IIS-Fundación Jiménez Díaz, Madrid, Spain. Correspondence: Carmen Garcés (cgarces@fjd.es) [2] Department of Pediatrics, IIS-Fundación Jiménez Díaz, Madrid, Spain [3] Social Epidemiology Research Unit, National Human Genome Research Institute, Bethesda, Maryland, USA [4] Royal Children's Hospital, Melbourne, Australia.

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GPR19, recent results have questioned whether adropin binds to GPR19, and evidence has indicated that adropin may represent a protein with multiple functions, acting as a secreted factor and/or membrane protein (4). While early studies have focused on its expression in the liver, it is more abundantly expressed in the brain (3,5,6).

Adropin is a product of the energy homeostasis associated (*Enho*) gene that was identified in 2008 by Kumar et al. (3) investigating liver gene expression in mouse models of obesity. Recently, Gao et al. (7) suggested that adropin regulates signal transduction pathways controlling hepatic glucose metabolism in obese mice. Other studies have demonstrated that adropin improves lipid metabolism and enhances insulin sensitivity in mice (8,9) and rats (10), with current knowledge regarding adropin mainly coming from animal studies.

The relationship between adropin and metabolism has been investigated in several studies in humans that suggest that adropin may contribute to body weight regulation, glucose, and lipid homeostasis (4) . Butler et al. (11), investigating the association of plasma adropin concentration with obesity in adults, found that adropin correlated negatively with BMI and that it was associated with a better lipid profile. Lower levels of adropin also were reported in patients with metabolic syndrome (12); however, the relationship between adropin and insulin resistance suggested in mice has not been clearly defined in humans (2,4,13,14). The relationship between adropin, obesity, and metabolic alterations has also been investigated in pediatric populations (15-19), with inconclusive results to date.

The aim of our study was to investigate the relationship of plasma adropin concentrations with obesity in Spanish children of two different ages.

# Methods

## Participants

A total of 223 Spanish children from two age groups (prepubertal and pubertal) were included in the study. Participants in our study were selected from participants in a cross-sectional study designed to examine cardiovascular risk factors in Spain (20,21). In this cross-sectional study, children were selected by means of random cluster sampling in schools and stratified by sex and type of school. Children identified as having obesity within the two age groups were selected as cases for our current study as well as a similar number of age- and sex-matched children selected as controls. The prepubertal group was composed of children between 6 and 8 years and the pubertal group between 14 and 17 years. Unfortunately, no information on Tanner stage was available in our study, but information on age at menarche was available in order to classify the pubertal status of the girls. Nevertheless, all children reported by parents to be suffering from chronic disease, including precocious and delayed puberty, were excluded from the study. Written consent was obtained from all parents before their children were included in the study. The study protocol was approved by the Ethics Committee of Clinical Investigation of the Fundación Jiménez Díaz (PIC016-2019 FJD). The investigation fulfills the principles contained in the Declaration of Helsinki and subsequent reviews as well as the prevailing Spanish legislation on clinical research in human participants.

## Anthropometric data

Measurements (weight and height) were taken with children wearing light clothing and barefoot. Height was measured to the millimeter by using a portable stadiometer, and weight was recorded to the nearest 0.1 kg by using a standard electronic digital scale. BMI (weight in kilograms/height in meters squared) was calculated from these measurements. BMI *z* score was also calculated according to Spanish reference data. Children were classified as having obesity if their BMI exceeded the age- and sex-specific cutoff points established for children by Cole et al. (22).

## Biochemical data

Blood samples were obtained early in the morning after a 12-hour fasting period by venipuncture into Vacutainer tubes that contained EDTA-Na2. Samples were centrifuged (1,500*g* at 4 °C for 25 minutes), and plasma was separated in aliquots, which were immediately stored at −80°C for further analysis. Cholesterol and triglycerides (TG) were determined enzymatically with a Technicon RA-1000 Autoanalyzer. The interassay coefficients of variation were 2.1% for cholesterol and 3.4% for TG. Insulin concentrations were measured by radioimmunoassay using a commercial kit (BI-Insulin IRMA, Bio-Rad). Insulin resistance was estimated using the homeostasis model assessment score (fasting insulin [μU/mL] × fasting glucose [mmol/L]/22.5). Leptin levels were determined by enzyme-linked immunosorbent assay (ELISA), using a commercially available kit (Leptin EIA-2395, DRG Instruments), with intra- and interassay coefficients of variation of 6.9% and 8.6%, respectively. Adropin concentration was measured by using a human adropin ELISA kit (Adropin CSB-EL007669HU, Cusabio), and intra- and interassay coefficients of variation were 2.8% and 5.5%, respectively.

## Statistical analysis

Statistical analysis was performed using the SPSS Statistics software package, version 21.0 (IBM) and GraphPad Prism statistical software, version 6. The normality of distribution of the variables under study was examined by using the Kolmogorov–Smirnov test. Variables that were not normally distributed (weight, BMI, TG, insulin, homeostasis model assessment, and leptin) were log-transformed before analysis. When data log-transformation failed to produce normal distribution (weight and BMI), nonparametric tests were used. Intergroup comparisons of means were performed by using the Student *t* test and Mann-Whitney *U* test depending on the distribution of the data. Univariate ANOVA was used to evaluate the association between adropin levels and the biochemical variables adjusting for BMI and gender. Associations between adropin and log-transformed leptin concentrations were evaluated using Pearson correlations. All comparisons were two-sided at a 0.05 significance level.

# Results

Our sample included the following two populations: 140 prepubertal children (71 with obesity and 69 with normal weight) and 83 pubertal children (41 with obesity and 42 with normal weight). The average ages of the children were 7.2 (0.6) years and 14.6 (0.9) years in prepubertal and pubertal children, respectively.

Adropin concentrations in children by age, sex, and weight category are shown in Figure 1. Regardless of weight category, adropin levels were significantly higher in prepubertal girls than in prepubertal boys (2.66 [0.17] ng/mL and 2.52 [0.25] ng/mL, respectively; $P < 0.01$),

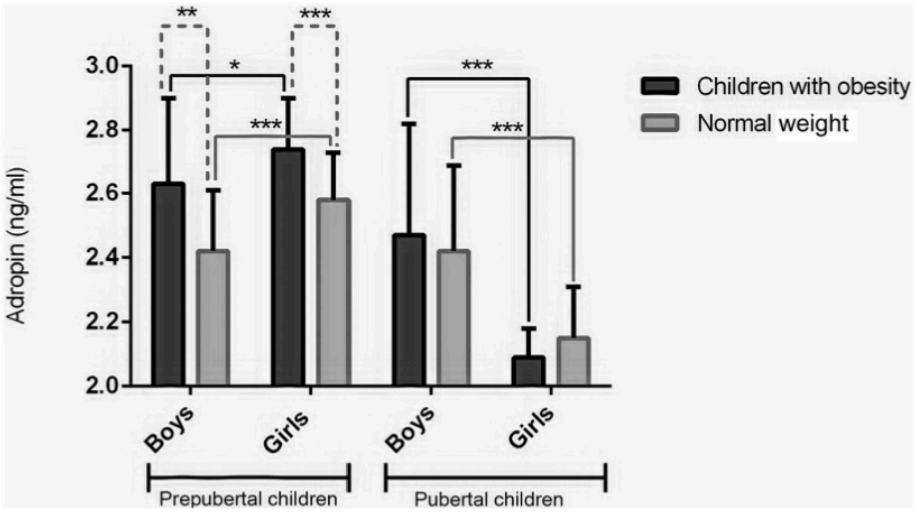


**Figure 1** Plasma adropin levels (nanograms per milliliter) in prepubertal and pubertal children by sex and weight category. *$P$=0.05, **$P$<0.01, ***$P$<0.001. $P$ values by Student $t$ test.

and, in contrast, adropin levels were significantly lower in pubertal girls than in pubertal boys (2.12 [0.13] ng/mL and 2.44 [0.31] ng/mL, respectively; $P$<0.001). Moreover, when analyzing children as a whole, plasma adropin levels were significantly higher ($P$<0.001) in prepubertal than in pubertal children (2.60 [0.22] ng/mL vs. 2.29 [0.29] ng/mL).

When analyzing plasma adropin concentrations by weight category in boys and girls by age group, we observed that prepubertal boys and girls with obesity had significantly higher adropin levels than their normal weight counterparts ($P$<0.01 and $P$<0.001, respectively). Moreover, no differences were observed in adropin levels between pubertal boys and girls with obesity and their normal weight counterparts (Figure 1).

To further analyze the relationship between adropin and anthropometric variables, and to investigate the relationship between adropin and lipid profile as well as insulin and leptin levels, plasma adropin was categorized into two groups (low and high adropin) by using the median value in each age group as cutoff point (Table 1). In the prepubertal age group, children with low adropin levels had significantly lower ($P$<0.001)

**TABLE 1** Anthropometric and biochemical variables in low- and high-adropin subgroups in prepubertal and pubertal children

| | Prepubertal | | | Pubertal | | |
|---|---|---|---|---|---|---|
| | Low (2.23-2.56) ($n$=72) | High (2.57-3.40) ($n$=68) | $P$ value | Low (1.94-2.23) ($n$=42) | High (2.24-3.59) ($n$=41) | $P$ value |
| Gender (M/F, %) | 60/40 | 28/72 | | 24/76 | 83/17 | |
| Age (y) | 7.2 (0.6) | 7.2 (0.5) | NS | 14.3 (1.3) | 14.1 (0.6) | NS |
| Birth weight (kg) | 3.33 (0.56) | 3.29 (0.57)) | NS | 3.21 (0.57) | 3.33 (0.50) | NS |
| Weight (kg) | 27.50 (7.81) | 32.69 (7.67) | <0.001 | 66.25 (20.77) | 72.22 (25.26) | NS |
| BMI (kg/m$^2$) | 17.16 (3.75) | 20.22 (3.71) | <0.001 | 24.85 (7.47) | 25.18 (7.67) | NS |
| BMI $z$ score | 0.11 (1.50) | 1.42 (1.40) | <0.001 | 0.91(2.15) | 1.03 (2.07) | NS |
| Total cholesterol (mg/dL) | 180.73 (25.17) | 181.32 (28.94) | NS | 166.67 (28.98) | 159.69 (29.15) | NS |
| Log-triglycerides (mg/dL) | 1.88 (0.13) | 1.89 (0.15) | NS | 1.86 (0.17) | 1.88 (0.21) | NS |
| HDL cholesterol (mg/dL) | 54.41 (11.80) | 54.27 (15.72) | NS | 50.62 (13.13) | 48.43 (15.15) | NS |
| LDL cholesterol (mg/dL) | 108.40 (23.39) | 110.58 (25.53) | NS | 100.40 (25.52) | 94.09 (25.59) | NS |
| Glucose (mg/dL) | 91.00 (8.78) | 92.16 (11.59) | NS | 91.86 (12.78) | 91.53 (11.05) | NS |
| Log-insulin (μU/mL) | 0.43 (0.34) | 0.55 (0.37) | NS | 0.98 (0.26) | 0.96 (0.21) | NS |
| Log-HOMA | −0.22 (0.36) | −0.10 (0.38) | NS | 0.33 (0.26) | 0.31 (0.22) | NS |
| Log-leptin (ng/mL) | 0.34 (0.57) | 0.76 (0.49) | 0.001 | 1.04 (0.60) | 0.77 (0.59) | 0.021 |

Data presented as mean (SD).
$P$ values by two-tailed unpaired Student $t$ test or values by Mann-Whitney $U$ test (weight, BMI, and BMI $z$ score).
F, female; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; M, male, NS, not significant.

values of weight, BMI, and BMI *z* score. Children in this group also had significantly lower ($P<0.001$) mean leptin levels than children in the high-adropin group. In the pubertal group, no differences for mean values of weight, BMI, and BMI *z* score were observed between the low- and high-adropin groups; however, in contrast to the prepubertal age group, mean leptin levels were significantly lower ($P<0.05$) in the high-adropin group than in the low-adropin group (Table 1). No significant differences were found for any of the other studied variables in either of the two age groups (Table 1). The associations between adropin and leptin levels remained the same after adjusting for BMI. After adjusting for sex, in the prepubertal group, mean log-leptin levels remained significantly lower in the low-adropin group than in the high-adropin group (0.35 ng/mL [95% CI: 0.22-0.48] and 0.75 ng/mL [95%CI: 0.62-0.89], respectively; $P<0.001$). In contrast, in the pubertal group, no statistically significant differences were observed between log-leptin levels in the low-adropin and in the high-adropin group (0.87 ng/mL [95% CI: 0.67-1.08] and 0.92 ng/mL [95% CI: 0.73-1.12], respectively; $P=0.746$).

When analyzing the association between adropin and leptin concentrations separately in males and females in each age group (Figure 2), positive associations were observed in prepubertal boys and girls, a negative association was observed in pubertal girls, and no association was observed in pubertal boys. Pearson correlation analysis showed significant positive correlations in prepubertal children (0.475, $P<0.001$ and 0.355, $P<0.01$ in boys and girls, respectively), a negative correlation in pubertal girls (–0.333, $P<0.059$), and no significant correlation in pubertal boys.

## Discussion

A potential role of adropin in metabolism homeostasis was initially suggested based on studies analyzing the liver expression of the *Enho* gene in obese mice (3). Later studies in humans have shown a negative association of adropin concentrations with BMI in adults (11,12,23) while finding positive, negative, or no associations in children (16-18,24). Our findings analyzing plasma adropin concentrations in two well-defined cohorts of prepubertal and pubertal children contribute to clarify the confusing relationship of adropin with obesity in children as we report several important observations. First, we described significantly lower adropin concentrations in pubertal than in prepubertal

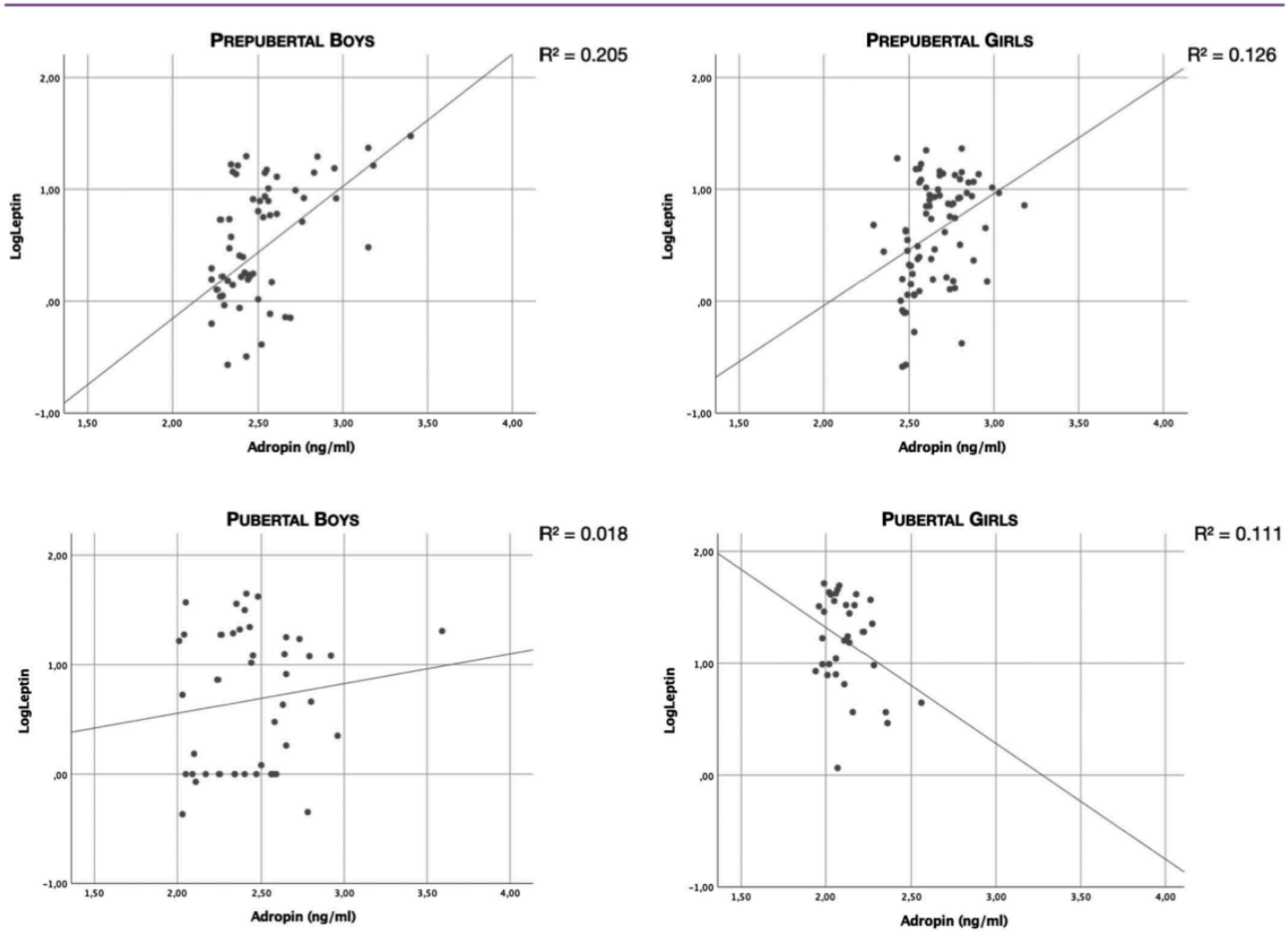


**Figure 2** Scatterplot showing associations between adropin concentrations and log-transformed leptin in prepubertal and pubertal children by sex.

children. Second, we described different adropin concentrations by sex, with opposite tendencies depending on age. Third, we found significantly higher adropin concentrations in prepubertal children with obesity than in their normal weight counterparts, without finding any difference between weight categories in pubertal children.

The finding of lower adropin concentrations in pubertal compared with prepubertal children is consistent with previously published data (11). Negative correlations between adropin and age have been described in studies in adults (11,25). However, the cause for this decline with age remains unclarified. Studies in rats have suggested that low plasma adropin concentration is associated with aging and endothelial dysfunction (26).

Prepubertal boys in our study had lower plasma adropin levels than girls. In contrast, pubertal boys had higher adropin concentrations than girls independent of weight category. In support of our findings in pubertal children, higher levels of adropin in men compared with women were previously described (11). The reason for this sex difference is unclear at this time. However, this opposite association between sex and adropin depending on age, along with our finding of significantly lower adropin levels in pubertal than in prepubertal children, suggests a potential influence of centrally regulated sex hormones involved in pubertal development on adropin secretion or the existence of different adropin neuronal populations according to sex and age.

The most startling finding in our study is the association of adropin with obesity and BMI in prepubertal children, which differed from the association found in pubertal children. Adropin levels were higher in prepubertal boys and girls with obesity than in their normal weight counterparts, but there was no significant difference in adropin levels when comparing pubertal children with and without obesity.

Adropin is a product of the *Enho* gene, mainly expressed in the brain in humans (5,27), and its expression in adipose tissue is very low (5). The expression of *Enho* and secretion of adropin by the liver were reported to be involved in peripheral lipid metabolism homeostasis in response to macronutrient consumption (3). Studies in humans have shown the association of adropin concentrations with macronutrient consumption (28,29). This could involve paracrine effects on hepatocytes and also act as an endocrine signal of macronutrient consumption and energy status. Central expression may substantially contribute to plasma adropin levels and justify an important role of centrally secreted adropin in metabolism.

In prepubertal children, we observed a positive association between adropin and leptin levels, while an inverse association was observed in pubertal children. Leptin is secreted in response to an excess of adipose tissue, with an appetite suppressant effect on the hypothalamus. Previous research found that, beyond its regulation of metabolism, appetite, and weight through neurons, leptin also acts on other types of cells to control appetite (30). Our hypothesis is that part of leptin's neuroendocrine role in the control of appetite may involve the regulation of adropin. Moreover, the hypothalamus and the pituitary are important endocrine glands that release key hormones in the regulation of development that could also be implicated in the regulation of the secretion of adropin. In prepubertal children, the positive correlation between leptin and adropin would be evident, while in pubertal children, other factors associated with sexual development could be significantly contributing to adropin secretion.

In our study, we failed to find any association of adropin with insulin levels or lipid profile. Studies in adults have demonstrated the relationship of adropin with insulin resistance (11) and metabolic syndrome (12). Furthermore, studies in mammalian cell lines have reported that adropin activates signaling pathways related to insulin sensitivity (31), with adropin regulating endothelial function (31). Adropin may be involved in the regulation of endothelial nitric oxide synthase bioactivity and endothelial function, known mediators of insulin sensitivity (26,31). Other neuroendocrine factors that regulate energy homeostasis and insulin sensitivity, such as adiponectin, also were demonstrated to modulate endothelial function (32). Likewise, resistin, a molecule known to inhibit insulin sensitivity, was shown to have deleterious effects on endothelial function (33). The lack of association between adropin and insulin sensitivity in the children in our study could be related to the fact that no alterations in endothelial function are likely to be found at these ages. Data regarding the association of adropin with lipid levels in humans are limited. Butler et al. (11,12,23) only found an association between adropin and TG after controlling for BMI. Ghoshal et al. (34) described a sex-dependent association between adropin and low-density lipoprotein (LDL) cholesterol and suggested an interaction between plasma adropin concentration and obesity in determining LDL cholesterol. The case-control design in our study, specifically designed to analyze the association of obesity with adropin, may have impacted our ability to demonstrate a significant association between adropin and lipid profile. Further studies are needed to investigate this association in children.

An important limitation in our study, as well as in the other studies determining adropin concentration, is the use of assays to determine adropin concentration that utilize adropin antibodies that may not be well validated. This makes it difficult to compare results between studies using different assays. Further efforts are required to develop methods to determine blood adropin concentrations to enable comparison between studies.

In summary, we described a differing association between obesity and adropin concentrations depending on age as well as an opposite direction of the association of adropin levels between sexes in prepubertal and in pubertal children. These findings suggest that adropin secretion may be related to hormones released by the hypothalamus that are implicated in the regulation of sexual development.O

## Acknowledgments

The article is dedicated to the late Prof. Manuel de Oya as the warmest homage to his memory. Prof. de Oya designed the Four Province Study, and the ideas reflected in our work can be traced back to his.

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## References

1. Ahima RS, Osei SY. Adipokines in obesity. *Front Horm Res* 2008;36:182-197.
2. Aydin S. Three new players in energy regulation: preptin, adropin and irisin. *Peptides* 2014;56:94-110.

3. Kumar KG, Trevaskis JL, Lam DD, et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. *Cell Metab* 2008;8:468-481.
4. Jasaszwili M, Billert M, Strowski MZ, Nowak KW, Skrzypski M. Adropin as a fat-burning hormone with multiple functions-review of a decade of research. *Molecules* 2020;25. doi:10.3390/molecules25030549
5. Butler AA, Zhang J, Price CA, et al. Low plasma adropin concentrations increase risks of weight gain and metabolic dysregulation in response to a high-sugar diet in male nonhuman primates. *J Biol Chem* 2019;294:9706-9719.
6. Wong CM, Wang Y, Lee JT, et al. Adropin is a brain membrane-bound protein regulating physical activity via the NB-3/notch signaling pathway in mice. *J Biol Chem* 2014;289:25976-25986.
7. Gao S, Ghoshal S, Zhang L, et al. The peptide hormone adropin regulates signal transduction pathways controlling hepatic glucose metabolism in a mouse model of diet-induced obesity. *J Biol Chem* 2019;294:13366-13377.
8. Ganesh Kumar K, Zhang J, Gao S, et al. Adropin deficiency is associated with increased adiposity and insulin resistance. *Obesity (Silver Spring)* 2012;20:1394-1402.
9. Gao S, McMillan RP, Zhu Q, Lopaschuk GD, Hulver MW, Butler AA. Therapeutic effects of adropin on glucose tolerance and substrate utilization in diet-induced obese mice with insulin resistance. *Mol Metab* 2015;4:310-324.
10. Akcılar R, Emel Koçak F, Şimşek H, et al. The effect of adropin on lipid and glucose metabolism in rats with hyperlipidemia. *Iran J Basic Med Sci* 2016;19:245-251.
11. Butler AA, Tam CS, Stanhope KL, et al. Low circulating adropin concentrations with obesity and aging correlate with risk factors for metabolic disease and increase after gastric bypass surgery in humans. *J Clin Endocrinol Metab* 2012;97:3783-3791.
12. Yosaee S, Khodadost M, Esteghamati A, et al. Metabolic syndrome patients have lower levels of adropin when compared with healthy overweight/obese and lean subjects. *Am J Mens Health* 2017;11:426-434.
13. Yildirim B, Celik O, Aydin S. Adropin: a key component and potential gatekeeper of metabolic disturbances in policystic ovarian syndrome. *Clin Exp Obstet Gynecol* 2014;41:310-312.
14. Wu L, Fang J, Chen L, et al. Low serum adropin is associated with coronary atherosclerosis in type 2 diabetic and non-diabetic patients. *Clin Chem Lab Med* 2014;52:751-758.
15. Altincik A, Sayin O. Evaluation of the relationship between serum adropin levels and blood pressure in obese children. *J Pediatr Endocrinol Metab* 2015;28:1095-1100.
16. Chang JB, Chu NF, Lin FH, Hsu JT, Chen PY. Relationship between plasma adropin levels and body composition and lipid characteristics amongst young adolescents in Taiwan. *Obes Res Clin Pract* 2018;12:101-107.
17. Kocaoglu C, Buyukinan M, Erdem SS, Ozel A. Are obesity and metabolic syndrome associated with plasma adropin levels in children? *J Pediatr Endocrinol Metab* 2015;28:1293-1297.
18. Sayın O, Tokgöz Y, Arslan N. Investigation of adropin and leptin levels in pediatric obesity-related nonalcoholic fatty liver disease. *J Pediatr Endocrinol Metab* 2014;27:479-484.
19. Zhang H, Jiang L, Yang Y-J, et al. Aerobic exercise improves endothelial function and serum adropin levels in obese adolescents independent of body weight loss. *Sci Rep* 2017;7:17717. doi:10.1038/s41598-017-18086-3
20. Garcés C, Gutierrez-Guisado J, Benavente M, et al. Obesity in spanish schoolchildren: relationship with lipid profile and insulin resistance. *Obes Res* 2005;13:959-963.
21. Schoppen S, Riestra P, García-Anguita A, et al. Leptin and adiponectin levels in pubertal children: relationship with anthropometric variables and body composition. *Clin Chem Lab Med* 2010;48:707-711.
22. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000;320:1240-1243.
23. Zang H, Jiang F, Cheng X, Xu H, Hu X. Serum adropin levels are decreased in Chinese type 2 diabetic patients and negatively correlated with body mass index. *Endocr J* 2018;65:685-691.
24. Orsso CE, Butler AA, Muehlbauer MJ, et al. Obestatin and adropin in Prader-Willi syndrome and nonsyndromic obesity: associations with weight, BMI-z, and HOMA-IR. *Pediatr Obes* 2019;14:e12493. doi:10.1111/ijpo.12493
25. Lian W, Gu X, Qin Y, Zheng X. Elevated plasma levels of adropin in heart failure patients. *Intern Med* 2011;50:1523-1527.
26. Yang C, DeMars KM, Candelario-Jalil E. Age-dependent decrease in adropin is associated with reduced levels of endothelial nitric oxide synthase and increased oxidative stress in the rat brain. *Aging Dis* 2018;9:322-330.
27. Marczuk N, Cecerska-Heryć E, Jesionowska A, Dołęgowska B. Adropin-physiological and pathophysiological role. *Postep Hig Med Dosw* 2016;70:981-988.
28. St-Onge MP, Shechter A, Shlisky J, et al. Fasting plasma adropin concentrations correlate with fat consumption in human females. *Obesity (Silver Spring)* 2014;22:1056-1063.
29. Stevens JR, Kearney ML, St-Onge MP, et al. Inverse association between carbohydrate consumption and plasma adropin concentrations in humans. *Obesity (Silver Spring)* 2016;24:1731-1740.
30. Kim JG, Suyama S, Koch M, et al. Leptin signaling in astrocytes regulates hypothalamic neuronal circuits and feeding. *Nat Neurosci* 2014;17:908-910.
31. Lovren F, Pan Y, Quan A, et al. Adropin is a novel regulator of endothelial function. *Circulation* 2010;122:S185-S192.
32. Ouchi N, Ohishi M, Kihara S, et al. Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension* 2003;42:231-234.
33. Verma S, Li SH, Wang CH, et al. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. *Circulation* 2003;108:736-740.
34. Ghoshal S, Stevens JR, Billon C, et al. Adropin: An endocrine link between the biological clock and cholesterol homeostasis. *Mol Metab* 2018;8:51-64.

## OBJETIVO 2:

## ESTUDIAR EL PAPEL DE PROTEÍNA C-REACTIVA (PCR), EN POBLACIÓN PEDIÁTRICA, COMO MARCADOR DE INFLAMACIÓN CRÓNICA Y DE ALTERACIONES METABÓLICAS E INVESTIGAR LOS FACTORES RELACIONADOS CON SUS NIVELES PLASMÁTICOS.

## OBJETIVO 2.1. INVESTIGAR LA POSIBLE EXPRESIÓN DE PCR EN TEJIDO ADIPOSO EN POBLACIÓN PEDIÁTRICA SOMETIDA A UNA APENDECTOMÍA.

Referencia:

**de Dios O**, Gavela-Pérez T, Aguado-Roncero P, Pérez-Tejerizo G, Ricote M, González, Garcés C, Soriano-Guillén L. C-reactive protein expression in adipose tissue of children with acute appendicitis. Pediatr Res 2018; 84: 564–567.


### RESUMEN

Objetivo: El objetivo de este estudio era conocer el papel del tejido adiposo visceral como fuente de proteína C-reactiva (PCR) en la inflamación aguda y explorar la posible relación de la expresión de PCR con la gravedad de la apendicitis.

Métodos: En el estudio se incluyó un total de 20 pacientes de edad pediátrica sometidos a apendicetomía. Los pacientes se dividieron en dos grupos según la gravedad de la apendicitis (sin complicaciones y con complicaciones). Los niveles de PCR se midieron tanto en muestras de grasa visceral mediante western-blot, como en suero mediante pruebas bioquímicas.

Resultados: Se encontró expresión de PCR en el tejido adiposo visceral. La expresión de PCR en tejido adiposo fue significativamente más elevada en pacientes con apendicitis complicada ($p = 0,002$) que en pacientes con apendicitis no complicada. Estos mismos resultados se reflejaron en los valores serológicos de PCR ($p = 0,018$).

Conclusión: Ya en la infancia, se encuentra expresión de PCR en tejido adiposo visceral y su expresión está potencialmente asociada con la gravedad de la inflamación local.

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Pediatric RESEARCH

BASIC SCIENCE ARTICLE

# C-reactive protein expression in adipose tissue of children with acute appendicitis

Olaya de Dios[1], Teresa Gavela-Pérez[1,2], Pablo Aguado-Roncero[3], Gloria Pérez-Tejerizo[3], Marta Ricote[4], Nieves González[4,5], Carmen Garcés[1] and Leandro Soriano-Guillén[1,2]

**OBJECTIVE:** The aim of this study is to gain insights into the role of visceral adipose tissue as a source of C-reactive protein (CRP) in acute inflammation and to explore the potential relationship of CRP expression with the severity of appendicitis.
**METHODS:** A total of 20 pediatric patients undergoing appendectomy were included in the study. Patients were divided into two groups according to appendicitis severity (uncomplicated and complicated). CRP levels were measured in visceral fat samples by western blotting, as well as in serum by biochemical testing.
**RESULTS:** CRP was found to be expressed in visceral adipose tissue. The adipose tissue of patients with complicated appendicitis showed significantly higher CRP levels ($p = 0.002$) compared to patients with uncomplicated appendicitis. These results mirrored the CRP values obtained in serum ($p = 0.018$).
**CONCLUSION:** In childhood, visceral adipose tissue is a source of CRP in acute inflammation, and its expression is potentially associated with the severity of local inflammation.

*Pediatric Research* (2018) 84:564–567; https://doi.org/10.1038/s41390-018-0091-z

## INTRODUCTION

C-reactive protein (CRP), which was discovered in 1930 in patients with pneumonia,[1] was the first acute-phase protein to be described. The acute phase is a reaction of the organism to bacterial, viral, or parasitic infection, trauma, ischemic necrosis, or malignant growth.[2] It is characterized by a non-specific systematic response of early onset (12–24 h), which causes an increase in the synthesis of acute-phase proteins.[3] In this type of response, CRP levels can be increased up to 1000 times and have an approximate average half-life of 19 h.[4]

As a result of this increased presence, CRP has been widely used in the clinic as a marker of acute inflammation,[2] though its characteristics also qualify it as a biomarker of the risk of acute appendicitis. There are studies on both children and adults that connect serum CRP levels with the severity of appendicitis, making the measurement of this protein a potential tool for the detection of complications.[5,6]

CRP is also considered as a marker of low-grade chronic inflammation in light of findings from studies carried out in recent years connecting CRP levels with body mass index.[7–9] In addition, CRP has been employed as a cardiovascular risk marker.[10,11,19] In the context of CRP as a marker of non-infectious inflammation, although adipose tissue has long been considered a mediator of hepatic CRP production due to the action of cytokines,[7] recent studies have shown that adipose tissue may be an extrahepatic source of CRP in humans.[8,9,12–14]

To the best of our knowledge, no studies performed to date have investigated CRP expression in the adipose tissue of children or the potential link between CRP production and the severity of an inflammatory process. Thus, the aims of this study were to gain insights into the role of visceral adipose tissue as a source of CRP in acute inflammation, and to explore the potential relationship of CRP expression with appendicitis severity.

## SUBJECTS AND METHODS

### Sample size calculation

There are no similar studies in the literature that can be used to obtain reference values, thus making this a pilot study. Assuming a theoretical value of one arbitrary unit in the group of uncomplicated appendicitis and with the hope of obtaining a greater than 100% difference in CRP expression in adipose tissue among the group with complicated appendicitis, for a β-statistical power of 80% and a level of α significance of 5%, at least six patients in each group would be needed.

### Study participants

Patients admitted to a pediatric hospital with a diagnosis of acute appendicitis and who underwent surgery were prospectively included in the study. The appendicitis diagnosis was established preoperatively by one of the consultant pediatric surgeons based on clinical history and physical examination. The patients were divided into two groups according to their anatomopathological findings.[5,15] The first group (group 1) comprised patients with uncomplicated appendicitis (an intact appendiceal mucosa with mild-to-moderate infiltration of inflammatory cells), and the second group (group 2) included patients with complicated appendicitis (perforated or gangrenous appendicitis).

Complete clinical information was available for all enrolled patients, including data on the surgical intervention,

[1]Lipid Laboratory, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain; [2]Department of Pediatrics, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain; [3]Department of Pediatric Surgery, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain; [4]Renal, Vascular and Diabetes Laboratory, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain and [5]Spanish Biomedical Research Network in Diabetes and Associated Metabolic Disorders (CIBERDEM), Madrid, Spain
Correspondence: Leandro Soriano-Guillén (leandro.soriano@uam.es, LSoriano@fjd.es)

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anatomopathological findings, serum CRP levels, age, gender, weight, height, and Tanner stage. We calculated the body mass index of each patient as follows: [weight (kg)/height$^2$ (m)]. Additionally, we estimated the Z-score for body mass index (BMI) according to age- and gender-matched Spanish reference data.[16]

Patients with incomplete clinical information were not included. Those patients with known chronic inflammatory pathology (i.e., gastrointestinal or rheumatic) were also ruled out from the study.

### Human biological material

Serum samples were obtained from patients at the time of admission for surgery. Visceral fat samples were obtained from the greater omentum, through partial distal omentectomy using vascular ligation and sectioned directly with scissors without the use of an electric scalpel. Liver samples used as CRP-positive control were obtained following informed consent from adult patients undergoing bariatric surgery. All samples were cleaned and stored in a sterile container at −80 °C for further biomolecular assays.

### Protein preparation for western blotting

For total protein extraction, the tissue samples (500 µg) were homogenized at 4 °C in 1.25% Triton X-100, containing 250 mM sucrose, 20 mM Tris/HCl, 2.5 mM $MgCl_2$, 50 mM β-mercaptoethanol, 1.2 mM EGTA, 1 mM $Na_3VO_4$, 5 mM $Na_4P_2O_7$, 50 mM NaF, 2 µM leupeptin, 2 µM pepstatin, pH 7.4, and 2 mM phenylmethylsulfonyl fluoride. The homogenized tissue samples were then maintained at 4 °C for 30 min and centrifuged at 15,000 × *g* to remove tissue debris. The supernatant (tissue lysate), containing cytosol and solubilized membrane proteins, was kept at −80 °C, and an aliquot of each extract was preserved for protein quantification by bicinchoninic acid assay (BCA, Thermo Fisher Scientific).[17]

### Electrophoresis and western blot assay

Equal amounts (30 µg) of tissue lysates from each sample were subjected to SDS-PAGE,[18,19] in parallel with molecular weight markers, on a 10% resolving gel. The resolved proteins were then transferred onto nitrocellulose membranes in a semidry system (Trans-Blot SD semi-dry transfer cell; Bio-Rad). For immunodetection, CRP antibody (1:1000) (Abcam, Y284) and β-actin antibody (1:2500) (Thermo Fisher Scientific, PA1-16889) were used as internal loading controls. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (1:2000) (Thermo Fisher Scientific, G21234) was employed as the secondary antibody. Proteins were visualized using the ECL kit (Thermo Fisher Scientific), with detection by the enhanced chemiluminescence method (GE Healthcare Bio-Sciences AB, ImageQuant LAS 4000), and analyzed with the image analysis program Quantity One (Bio-Rad Laboratories, Hercules, CA). Data are reported as the band intensity of immunostaining values (arbitrary units) obtained by normalization based on the densitometry value of the CRP relative to those obtained for β-actin. The value obtained from human liver tissue was used as a control.

### Determination of serum CRP concentrations

Serum samples were quantified by an autoanalyzer using an immunoassay based on an enzymatic heterogeneous, sandwich immunoassay format (Ortho-Clinical Diagnostics). The limit of detection for the assay was 0.272 mg/dl and the limit of quantitation was 0.480 mg/dl. Levels below the limit of quantitation were set to 0.5 mg/dl before statistical analysis.

### Statistical analysis

The SPSS 19.0 and GraphPad Prism statistical programs were used for statistical analysis. We used the Kolmogorov–Smirnov test to analyze the normality of the variables studied. Variables not normally distributed were log-transformed before analysis. The data were expressed as means ± SEM (standard error of mean) and analyzed by a parametric Student's *t*-test. Categorical variables were compared using the chi-square test. Statistical significance was set at a *p*-value <0.05.

### Ethical approval

Informed written consent was obtained from parents or legal guardians of all participants, but assent was also obtained from patients older than 12 years before being enrolled in the study. The study was approved by the Ethics Committee of the Instituto de Investigación Sanitaria-Fundación Jiménez Díaz, Madrid, Spain, in accordance with the Declaration of Helsinki.

## RESULTS

A total of 20 patients were studied, of whom 11 belonged to group 1 (uncomplicated AA) and nine to group 2 (complicated AA). There were no significant differences in gender, age, Z-score, BMI, or Tanner stage (Table 1).

CRP expression was confirmed in the visceral adipose tissue of these children, particularly in patients with complicated appendicitis. To analyze the relationship between CRP expression levels and the severity of appendicitis, the averages of the arbitrary CRP units of groups 1 and 2 were compared (uncomplicated appendicitis vs. complicated appendicitis), revealing a significant difference between the two groups ($p = 0.002$). The lowest expression levels were detected in group 1 (0.96 ± 0.68), while the highest values were seen in group 2 (4.11 ± 1.20) (Fig. 1).

Lower serum CRP levels were found in patients from group 1 (2.86 ± 0.89 mg/dl) compared to those from group 2 (10.33 ± 2.71 mg/dl), with this difference reaching statistical significance ($p = 0.018$) (Fig. 2). In the non-complicated appendicitis group, 5 out of the 11 patients presented serum CRP levels below 0.5 mg/dl, whereas in the complicated appendicitis group, only one patient out of nine presented levels lower than 0.5 mg/dl.

## DISCUSSION

The present study demonstrates CRP expression at the protein level in visceral adipose tissue of children with appendicitis. Previous studies have evidenced the expression of CRP in adipose tissue at both the protein and mRNA level;[12–16] to our knowledge, our study is the first to show protein-level expression in samples from pediatric patients with appendicitis.

We have also found out that there was a significant difference between the expression of CRP in adipose tissue of patients with uncomplicated and complicated appendicitis, revealing fourfold higher values in those with complicated appendicitis. Thus, CRP expression in visceral adipocytes could be linked to the degree of local inflammation, which likely triggers the inflammatory response. Our findings support those of Peyrin-Biroulet et al.,[9] as in their study on CRP in Crohn's disease, the authors concluded

**Table 1.** Characteristics of the patients

| | Patients | | *p*-value |
|---|---|---|---|
| | Group 1 ($n = 11$) | Group 2 ($n = 9$) | |
| Gender (male/female) | 4/7 | 7/2 | NS |
| Age | 13.17 ± 1.23 | 12.32 ± 1.66 | NS |
| Z-score BMI | −0.33 ± 0.29 | −0.27 ± 0.20 | NS |
| Tanner stage (prepubertal/pubertal) | 3/8 | 3/6 | NS |

NS indicates not statistically significant

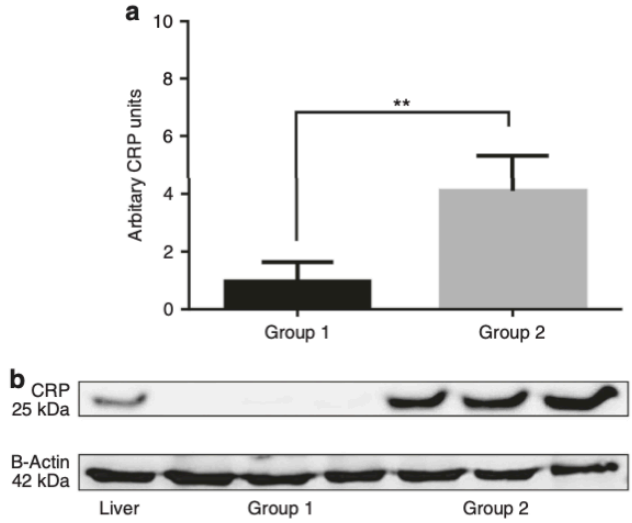


**Fig. 1** **a** Comparison of CRP expression measured by western blot in the visceral fat of patients with uncomplicated appendicitis (group 1) and complicated appendicitis (group 2). **b** CRP expression was measured by western blot analysis. This image is representative of the experiments and showed one sample of liver tissue (CRP-positive control) and six samples of visceral adipose tissue from patients with appendicitis undergoing surgery. The first three samples belong to group 1 (uncomplicated appendicitis) and the next three to group 2 (complicated appendicitis). Actin protein levels were determined as an internal loading control. The results are expressed as means ± SEM. ***p*-values by Student's *t* test: <0.01

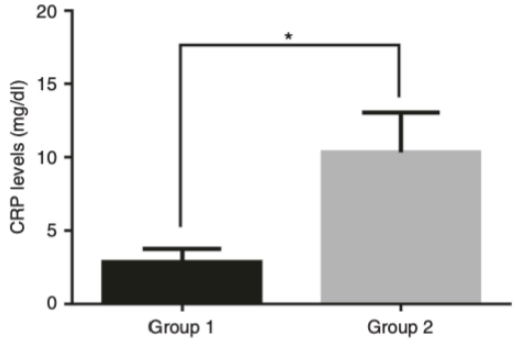


**Fig. 2** Comparison of mean serum CRP levels in patients with uncomplicated appendicitis (group 1) vs. complicated appendicitis (group 2) as measured by an autoanalyzer. The results are expressed as means ± SEM. **p*-values by Student's *t* test: <0.05

that the inflammatory response is likely triggered due to the production of CRP resulting from local inflammation of adipocytes.

The fact that adipose tissue has a greater expression of CRP in cases of complicated than uncomplicated appendicitis is of great relevance, as it not only confirms that adipose tissue is an extrahepatic source of CRP, but also indicates that adipose tissue may actively participate in the inflammatory response by increasing the production of CRP. It should be noted that adipose tissue upregulates the expression of IL-6 and IL-6 receptors,[12] which play a fundamental role in regulating CRP expression, thereby increasing the synthesis and secretion of CRP by adipocytes.[12,13] Indeed, this study would have been enriched by an evaluation of CRP expression in adipose tissue in chronic low-grade inflammatory obesity, as recent studies have done.[12]

With regard to blood CRP levels, several studies, both in adults[6,20–22] and in children,[5,23,24] have shown that blood CRP levels are an accurate marker of appendicitis severity and therefore are a useful tool in the diagnosis of complicated appendicitis. Our results support these findings, as we observed a 3.6-fold increase in serum CRP levels at complicated appendicitis. In addition, 54.5% of patients with uncomplicated appendicitis had normal values of serum CRP (<0.5 mg/dl), which lends further support to the study of Grönroos et al.,[25] which found that normal CRP levels were not indicative of an absence of appendicitis in children.

Having considered the results obtained in adipose tissue and serum, we can observe the same behavior in both the cases, but CRP increases according to the severity of inflammation. However, methodological limitations prevented us from assessing the possible correlation between CRP levels in adipose tissue and serum.

The main limitation of our study lies in our inability to obtain control samples of adipose tissue from healthy patients, as such samples could have been used to test CRP protein expression in healthy patients. Another limiting factor is the absence of quantitative measurement CRP levels secreted by adipose tissue. It is possible that a bigger sample size would be of interest; however, consistent results have been found. Lastly, an analysis of the CRP expression levels in subcutaneous adipose tissue would have enriched our study.

In conclusion, visceral adipose tissue in pediatric age is a source of CRP in acute inflammation and its expression is potentially associated with the severity of local inflammation in appendicitis. These results set up future lines of research that could elucidate the role of adipose tissue and CRP in chronic non-infectious inflammatory processes.

## ACKNOWLEDGEMENTS

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## ADDITIONAL INFORMATION

**Competing interests:** Olaya de Dios is a fellow of the Conchita Rábago Foundation. The remaining authors declare that they have no competing interests.

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## REFERENCES

1. Tillett, W. S. & Francis, T. Serological reactions in pneumonia with a nonprotein somatic fraction of pneumococcus. *J. Exp. Med.* **52**, 561–571 (1930).
2. Jain, S., Gautam, V. & Naseem, S. Acute-phase proteins: as diagnostic tool. *J. Pharm. Bioallied Sci.* **3**, 118–127 (2011).
3. Black, S., Kushner, I. & Samols, D. C-reactive protein. *J. Biol. Chem.* **279**, 48487–48490 (2004).
4. Pepys, M. B. & Hirschfield, G. M. C-reactive protein: a critical update. *J. Clin. Invest.* **111**, 1805–1812 (2003).
5. Gavela, T., Cabeza, B., Serrano, A. & Casado-Flores, J. C-reactive protein and procalcitonin are predictors of the severity of acute appendicitis in children. *Pediatr. Emerg. Care* **28**, 416–419 (2012).
6. Buyukbese Sarsu, S. & Sarac, F. Diagnostic value of white blood cell and C-reactive protein in pediatric appendicitis. *Biomed. Res. Int.* **2016**, 6508619 (2016).
7. Therapy, E. A new perspective on the biology of C-reactive protein. *Circ. Res.* **3**, 12–15 (2005).
8. Noorjamal, N. A. et al. C-reactive protein is highly expressed in visceral adipose tissue of obese but not lean subjects: a post mortem study. *Atherosclerosis* **263**, e122 (2017).
9. Peyrin-Biroulet, L. et al. Mesenteric fat as a source of C reactive protein and as a target for bacterial translocation in Crohn's disease. *Gut* **61**, 78–85 (2012).
10. Greenfield, J. R. et al. Obesity is an important determinant of baseline serum C-reactive protein concentration in monozygotic twins, independent of genetic influences. *Circulation* **109**, 3022–3028 (2004).
11. Choi, J., Joseph, L. & Pilote, L. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. *Obes. Rev.* **14**, 232–244 (2013).

12. Memoli, B. et al. Inflammation may modulate IL-6 and C-reactive protein gene expression in the adipose tissue: the role of IL-6 cell membrane receptor. *Am. J. Physiol. Endocrinol. Metab.* **293**, E1030–E1035 (2007).
13. Anty, R. et al. The inflammatory C-reactive protein is increased in both liver and adipose tissue in severely obese patients independently from metabolic syndrome, type 2 diabetes, and NASH. *Am. J. Gastroenterol.* **101**, 1824–1833 (2006).
14. Ouchi, N. et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* **107**, 671–674 (2003).
15. Vaughan-Shaw, P. G., Rees, J. R., Bell, E., Hamdan, M. & Platt, T. Normal inflammatory markers in appendicitis: evidence from two independent cohort studies. *JRSM Short. Rep.* **2**, 43 (2011).
16. Carrascosa Lezcano, A. et al. [Spanish cross-sectional growth study 2008. Part II. Height, weight and body mass index values from birth to adulthood]. *An Pediatr.* **68**, 552–569 (2008).
17. Smith, P. K. et al. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76–85 (1985).
18. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685 (1970).
19. Oliveira, A. C. et al. Alanine aminotransferase and high sensitivity C-reactive protein: correlates of cardiovascular risk factors in youth. *J. Pediatr.* **152**, 337–342 (2008).
20. Kaya, B. et al. The diagnostic value of D-dimer, procalcitonin and CRP in acute appendicitis. *Int J. Med Sci.* **9**, 909–915 (2012).
21. Mcgowan, D. R., Sims, H. M., Zia, K., Uheba, M. & Shaikh, I. A. The value of biochemical markers in predicting a perforation in acute appendicitis. *ANZ J Surg* **83**, (79–83 (2013).
22. Panagiotopoulou, I. G. et al. The diagnostic value of white cell count, C-reactive protein and bilirubin in acute appendicitis and its complications. *Ann. R. Coll. Surg. Engl.* **95**, 215–221 (2013).
23. Chung, J. L. et al. Diagnostic value of C-reactive protein in children with perforated appendicitis. *Eur. J. Pediatr.* **155**, 529–531 (1996).
24. Zani, A. et al. Can common serum biomarkers predict complicated appendicitis in children? *Pediatr Surg Int* 33, (799–805 (2017).
25. Grönroos, P., Huhtinen, H. & Grönroos, J. M. Normal leukocyte count and C-reactive protein value do not effectively exclude acute appendicitis in children. *Dis. Colon Rectum* **52**, 1028–1029 (2009).

## OBJETIVO 2.2. EVALUAR LA UTILIDAD DE PCR COMO BIOMARCADOR DE SÍNDROME METABÓLICO INDEPENDIENTE DE LOS NIVELES DE LEPTINA Y ADIPONECTINA, EN LA COHORTE DE ADOLESCENTES DEL ESTUDIO 4P.

Referencia:

**de Dios O**, Vales-Villamarín C, Herrero L, Pérez-Segura P, Soriano-Guillén L, Garcés C. Analysis of leptin-adiponectin ratio and C-reactive protein as potential biomarkers of metabolic syndrome in adolescent. Clin Chem Lab Med.


### RESUMEN

Objetivos: El síndrome metabólico (SMet) se ha asociado con alteraciones endocrinas relacionadas con adiposidad e inflamación crónica; sin embargo, su asociación específica con moléculas relacionadas con el tejido adiposo o biomarcadores inflamatorios sigue sin estar clara, particularmente en poblaciones jóvenes. Nuestro objetivo fue investigar la relación de las concentraciones de leptina, adiponectina, el leptina/adiponectina y proteína C-reactiva de alta sensibilidad (as-PCR) con la presencia de SMet.

Métodos: La muestra del estudio estaba compuesta por 695 adolescentes (374 niñas y 321 niños). En todos ellos, se disponía de la información sobre parámetros antropométricos, presión arterial, glucosa, perfil lipídico y concentraciones de leptina, adiponectina y as-PCR. Se utilizó la definición de la Federación Internacional de Diabetes pediátrica para clasificar a los adolescentes con SMet.

Resultados: En ambos sexos, se observó un aumento progresivo y significativo de las concentraciones de leptina y del ratio leptina-adiponectina en los grupos estudiados (0 componentes de SMet, 1-4 componentes y SMet+). Las diferencias en los niveles de adiponectina y as-PCR entre grupos fueron menos importantes, en particular en niños. En el análisis de regresión logística, en niñas, tanto el ratio leptina-adiponectina como los niveles de as-PCR surgen como predictores significativos de la presencia de 1 a 4 componentes de SMet y de SMet+. En niños, únicamente el ratio leptina-adiponectina aparece como un predictor significativo de la presencia de 1-4 características de SMet y presencia de SMet+.

Conclusiones: Nuestros resultados muestran que el ratio leptina-adiponectina aparece como un biomarcador independiente de SMet en adolescentes, mientras que la asociación entre las concentraciones de as-PCR y SMet a la edad de los niños estudiados fue evidente únicamente en niñas.

Letter to the Editor

Olaya de Dios, Claudia Vales-Villamarín, Leticia Herrero, Pilar Pérez-Segura, Leandro Soriano-Guillén and Carmen Garcés*

# Analysis of leptin-adiponectin ratio and C-reactive protein as potential biomarkers of metabolic syndrome in adolescents

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**Keywords:** adolescents; C-reactive protein; leptin-adiponectin ratio; metabolic syndrome.

To the Editor,

Raising obesity rates in young population has led to an increase in the prevalence of metabolic syndrome (MetS) early in life [1]. Obesity is defined by excessive accumulation and dysfunction of adipose tissue, characterized by the secretion of adipose tissue-derivate proinflammatory cytokines that lead to the development of a cluster of disorders related to the pathogenesis of the MetS. Although the precise pathways behind this pathology remain under study, there is scientific evidence indicating that the MetS is an inflammatory disorder in which adipokines such as leptin and adiponectin play an important role [2] and the ratio of these two adipokines has been proposed as a good marker of adipose tissue dysfunction [3]. These adipokines have been related to MetS even in pediatric populations with studies suggesting that the leptin/adiponectin ratio may be a better marker for cardiometabolic risk in children with obesity than leptin and adiponectin concentrations separately [4].

C-reactive protein (CRP) is an accepted systemic marker for inflammation that has been linked to the presence of MetS as well as its features [2]. In this context, it is important to highlight that CRP levels have been closely related to leptin and adiponectin, and the combined actions between PCR and adipokines regarding metabolic alterations has been postulated [5]; therefore, an interaction between these metabolic and inflammatory markers in MetS pathogenesis could be assumed. However, to our knowledge, the relationship between these biomarkers and MetS has not been investigated in a general young population.

We aimed to analyze the relationships of leptin, adiponectin, leptin-adiponectin ratio and hs-CRP levels with the presence of MetS and the number of its components in a population-based sample including 695 adolescents (374 females and 321 males) aged 12–16 years. The pediatric International Diabetes Federation (IDF) definition was used to classify adolescents for MetS as previously described [6]. Participants were part of a cross-sectional study examining cardiovascular risk factors in Spanish schoolchildren, the Four Provinces Study, in whom information regarding leptin, adiponectin and hs-CRP concentrations was available [7, 8]. The study protocol was approved by the Ethics Committee of Clinical Investigation of the Instituto de Investigación Sanitaria-Fundación Jiménez Díaz (PIC016-2019 FJD) and fulfills with Helsinki Declaration guidelines and Spanish legislation on clinical research in human subjects. Parents or legal guardians were required to provide written consent for their children to participate in the study.

In our analysis, subjects were subdivided into three groups according to the number of IDF MetS features they presented: subjects not presenting any features: group 1; subjects presenting 1–4 features: group 2; and subjects with MetS$^+$ (central obesity plus any other two features): group 3.

A significant progressive increase in leptin concentrations and leptin-adiponectin ratio and a significant decrease in adiponectin from group 1 to group 3 were observed when comparing concentrations in group 1 (zero features), group 2 (1–4 features) and group 3 (MetS$^+$), with

***Corresponding author: Carmen Garcés**, PhD, Lipid Research Laboratory, IIS-Fundación Jiménez Díaz, Avda. Reyes Católicos, 2, 28040 Madrid, Spain, Phone: +34 91 5504892,
E-mail: cgarces@fjd.es
**Olaya de Dios, Claudia Vales-Villamarín and Leticia Herrero,** Lipid Research Laboratory, IIS-Fundación Jiménez Díaz, Madrid, Spain
**Pilar Pérez-Segura and Leandro Soriano-Guillén,** Department of Pediatrics, IIS-Fundación Jiménez Díaz, Madrid, Spain

**Table 1:** Leptin, adiponectin, leptin-adiponectin ratio (L/A ratio) and hs-CRP concentrations (mean [SD]), in males and females, according to the MetS groups.

| Males | Group 1 Zero features (n=151) | Group 2 1–4 features (n=159) | Group 3 MetS$^+$ (n=11) | p-Value 1–2 | p-Value 1–3 | p-Value 2–3 |
|---|---|---|---|---|---|---|
| Age, years | 14.3 (1.2) | 14.4 (1.2) | 14.5 (1.3) | NS | NS | NS |
| BMI, kg/m$^2$ | 20.6 (2.7) | 22.8 (4.1) | 30.6 (2.7) | [c] | [c] | [c] |
| z-score BMI | −0.16 (0.73) | 0.40 (1.12) | 2.39 (1.10) | [c] | [c] | [c] |
| Leptin, ng/mL | 4.1 (4.8) | 6.4 (7.5) | 16.5 (7.4) | [b] | [c] | [c] |
| Adiponectin, µg/mL | 12.2 (7.5) | 10.4 (5.7) | 6.8 (3.7) | NS | [b] | [a] |
| L/A ratio | 0.49 (0.67) | 0.89 (1.25) | 3.09 (1.70) | [c] | [c] | [c] |
| hs-CRP, mg/L | 0.63 (1.05) | 0.93 (1.31) | 1.67 (2.02) | [a] | [b] | NS |
| **Females** | **Group 1 Zero features (n=221)** | **Group 2 1–4 features (n=149)** | **Group 3 MetS$^+$ (n=4)** | **p-Value 1–2** | **p-Value 1–3** | **p-Value 2–3** |
| Age, years | 14.3 (1.0) | 14.4 (1.2) | 14.2 (1.6) | NS | NS | NS |
| BMI, kg/m$^2$ | 20.7 (2.3) | 23.1 (3.9) | 31.5 (9.4) | [c] | [c] | [c] |
| z-score BMI | −0.11 (0.62) | 0.55 (1.12) | 2.82 (2.76) | [c] | [c] | [c] |
| Leptin, ng/mL | 11.7 (7.4) | 17.7 (12.1) | 37.4 (14.5) | [c] | [c] | [a] |
| Adiponectin, µg/mL | 16.3 (8.1) | 13.8 (7.6) | 8.3 (1.5) | [a] | [b] | [a] |
| L/A ratio | 1.07 (1.40) | 1.90 (2.64) | 4.40 (1.40) | [c] | [c] | [a] |
| hs-CRP, mg/L | 0.50 (0.74) | 0.79 (1.28) | 2.54 (2.59) | [a] | [a] | [a] |

p-Value: Post Hoc Tukey or Games-Howell test; [a]p<0.05, [b]p<0.01, [c]p<0.001.

important differences in mean values between groups in both sexes particularly for leptin levels and leptin-adiponectin ratio (Table 1). Regarding hs-CRP, in females, significantly lower concentrations were observed in group 1 comparing with groups 2 and 3, and in group 2 comparing with group 3. In males, weaker differences in hs-CRP concentrations between groups were observed, with no significant differences between group 2 and group 3 (Table 1).

Logistic regression analysis including leptin-adiponectin ratio and hs-CRP in the model showed that, in males, only leptin-adiponectin ratio appeared as a significant predictor of both, the presence of between 1 and 4 features of MetS and the presence of MetS (Table 2). In females, both the leptin-adiponectin ratio and hs-CRP levels were significant predictors of having between 1 and 4 features of MetS and MetS$^+$ (Table 2).

When analyzing the correlations of hs-CRP with adipokines, in males, we observed positive correlations with leptin (r=0.360, p<0.001) and the leptin-adiponectin ratio (r=0.354, p<0.001), that remained significant after adjusting for BMI (leptin: r=0.132, p<0.05; leptin-adiponectin ratio: r=0.148, p<0.01). In females, we found weaker correlations between hs-CRP and leptin (r=0.195, p<0.001) and the leptin-adiponectin ratio (r=0.200, p<0.001), that were not significant after adjusting for BMI (leptin: r=0.033, p=0.395; leptin-adiponectin ratio: r=0.058, p=0.379). No significant correlations were found between hs-CRP and adiponectin in any sex.

Thus, in our study aimed to clarify the role of adipokines and hs-CRP as biomarkers of the MetS in adolescents,

**Table 2:** Relationship of leptin-adiponectin ratio (L/A ratio) and hs-CRP levels with the number of MetS components and the presence of MetS.

| Males | 1–4 Features OR | 1–4 Features 95%CI | 1–4 Features p-Value | MetS$^+$ OR | MetS$^+$ 95%CI | MetS$^+$ p-Value |
|---|---|---|---|---|---|---|
| L/A ratio | 1.62 | 1.19–2.20 | 0.002 | 3.07 | 1.98–4.76 | <0.0001 |
| hs-CRP, mg/L | 1.15 | 0.93–1.42 | 0.204 | 1.39 | 0.94–2.05 | 0.100 |
| **Females** | **1–4 Features OR** | **1–4 Features 95%CI** | **1–4 Features p-Value** | **MetS$^+$ OR** | **MetS$^+$ 95%CI** | **MetS$^+$ p-Value** |
| L/A ratio | 1.34 | 1.15–1.57 | <0.0001 | 1.50 | 1.19–1.88 | <0.0001 |
| hs-CRP, mg/L | 1.29 | 1.02–1.63 | 0.031 | 2.01 | 1.29–3.12 | 0.002 |

OR and 95% CI obtained by logistic regression analysis.

we found that the leptin-adiponectin ratio has a strong association with MetS or increased number of MetS components in both sexes, and, on the other hand, that hs-CRP concentrations have a weaker association with an accumulative number of MetS components or with the presence of MetS, to the point that, when taking into account the leptin-adiponectin ratio, the association is significant only in females.

The ratio between leptin and adiponectin has emerged as the better marker for MetS in both adults [3] and children [4]. The alteration of adiponectin and leptin secretion triggers the inflammatory response of adipose tissue, and the leptin-adiponectin ratio is considered a biomarker of dysfunctional adipose tissue contributing to the increased oxidative stress and inflammation that characterized the MetS [9]. On the other hand, an influence of CRP on leptin and adiponectin expression has been described [10], with CRP potentially affecting the functionality of these adipokines and, therefore, the alterations associated with MetS. As described in other studies [5] and reported previously in our population [8], we observed a correlation between hs-CRP concentrations and leptin and leptin-adiponectin ratio, that after adjusting for BMI remained significant only in males. It seems that, in males in our study, the association between hs-CRP concentrations and MetS is mediated by the leptin-adiponectin ratio as, after taking into account this ratio, no differences were found comparing hs-CRP levels in males with MetS or with several MetS components with hs-CRP levels in males without features of MetS. On the other hand, in females, in whom no correlation between and hs-CRP and leptin-adiponectin ratio is observed after adjusting by BMI, hs-CRP concentrations in the group with several MetS features and in those with MetS remain significantly higher than in the group of females without features of MetS after taking into consideration the leptin-adiponectin ratio. Our findings suggest an association of hs-CRP with MetS or its components at this age depending on sex. Nevertheless, further studies are needed to investigate these sex-related differences in the association of hs-CRP with MetS.

As limitations in our study, we must mention the lack of information on puberty precluding correction here for and the small number of cases encountered in the MetS + groups, which may influence the statistical power to detect differences in the studied variables between children with MetS and the other two groups. However, our cohort is a representative sample and reflects MetS distribution of the Spanish adolescent population. Anyhow, further studies with a higher number of children with MetS would be appropriate.

In conclusion, our results show that the leptin-adiponectin ratio is an independent biomarker of MetS in children from 12 to 16 years of age. The association between hs-CRP concentrations and MetS, however, is evident only in females. Our findings highlight the potential role of the leptin-adiponectin ratio as a useful diagnostic biomarker for MetS already at this age, helping to identify subjects who may require intervention to prevent cardiovascular events in the future.

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## References

1. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 2004;350:2362–74.
2. Reddy P, Lent-Schochet D, Ramakrishnan N, McLaughlin M, Jialal I. Metabolic syndrome is an inflammatory disorder: a conspiracy between adipose tissue and phagocytes. Clin Chim Acta 2019;496:35–44.
3. Frühbeck G, Catalán V, Rodríguez A, Gómez-Ambrosi J. Adiponectin-leptin ratio: a promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. Adipocyte 2018;7:57–62.
4. Frithioff-Bøjsøe C, Lund MAV, Lausten-Thomsen U, Hedley PL, Pedersen O, Christiansen M, et al. Leptin, adiponectin, and their ratio as markers of insulin resistance and cardiometabolic risk in childhood obesity. Pediatr Diabetes 2020;21:194–202.
5. Graßmann S, Wirsching J, Eichelmann F, Aleksandrova K. Association between peripheral adipokines and inflammation markers: a systematic review and meta-analysis. Obesity 2017;25:1776–85.
6. De Oya I, Schoppen S, Lasunción MA, Lopez-Simon L, Riestra P, de Oya M, et al. Sex hormone-binding globulin levels and

metabolic syndrome and its features in adolescents. Pediatr Diabetes 2010;11:188–94.
7. Schoppen S, Riestra P, García-Anguita A, López-Simón L, Cano B, de Oya I, et al. Leptin and adiponectin levels in pubertal children: relationship with anthropometric variables and body composition. Clin Chem Lab Med 2010;48:707–11.
8. Navarro P, de Dios O, Gavela-Pérez T, Jois A, Garcés C, Soriano-Guillén L. High-sensitivity C-reactive protein and leptin levels related to body mass index changes throughout childhood. J Pediatr 2016;178:178–82.
9. Frühbeck G, Catalán V, Rodríguez A, Ramírez B, Becerril S, Salvador J, et al. Involvement of the leptin-adiponectin axis in inflammation and oxidative stress in the metabolic syndrome. Sci Rep 2017;7:1–8.
10. Yuan G, Jia J, Di L, Zhou L, Dong S, Ye J, et al. Effects of C-reactive protein on adipokines genes expression in 3T3-L1 adipocytes. Biochem Biophys Res Commun 2012;424:462–8.

## OBJETIVO 2.3. DETERMINAR POSIBLES FACTORES RELACIONADOS CON LOS NIVELES PLASMÁTICOS DE PCR.

### OBJETIVO 2.3.1. INVESTIGAR LA POSIBLE ASOCIACIÓN ENTRE LOS VALORES SANGUÍNEOS DE PCR Y LOS NIVELES DE HORMONAS SEXUALES EN LA SEGUNDA COHORTE DEL ESTUDIO 4P.

Referencia:

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#### RESUMEN

Antecedentes: Se ha descrito una asociación entre las hormonas sexuales con los niveles de proteína C-reactiva (PCR). Sin embargo, esta asociación permanece inexplorada en niños en los que se están produciendo importantes cambios antropométricos y hormonales.

Objetivos: Analizar la asociación entre PCR de alta sensibilidad (as-PCR) y niveles de testosterona, estradiol y globulina transportadora de hormonas sexuales (SHBG) en una muestra de adolescentes y evaluar la influencia de los niveles de leptina en esta asociación.

Materiales y métodos: La muestra de este estudio transversal se compuso de 338 niños y 385 niñas, de edades entre los 12 y los 16 años, de los que se disponía de información sobre variables antropométricas y de los niveles de las hormonas sexuales, leptina y as-PCR.

Resultados: En los niños de nuestro estudio, la edad se asoció significativamente con niveles más elevados de testosterona y con concentraciones más bajas de leptina y SHBG. En las niñas, no se observaron cambios significativos según la edad en los niveles de leptina y SHBG. En los niños, la leptina se correlacionó negativamente con los niveles de testosterona (−0,263, $p < 0,001$), mostrando una correlación más fuerte después de ajustar por índice de masa corporal (IMC) (−0,424, $p < 0,001$). En los niños, también se observó una correlación significativa entre la as-PCR y los niveles de testosterona después de ajustar por IMC; sin embargo, la correlación desapareció después de ajustar por leptina. No se observó asociación entre testosterona y hs-PCR en niñas. La asociación negativa entre los niveles de as-PCR y SHBG siguió siendo significativa después del ajuste por leptina en ambos sexos, pero desapareció en los niños después del ajuste por IMC.

Conclusión: La asociación negativa entre la hs-PCR y las concentraciones de testosterona observada en niños de 12 a 16 años parece estar relacionada con los niveles de leptina, que están estrechamente relacionados de forma negativa con los valores de testosterona independientemente del IMC.

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# Sex steroid hormones, leptin, and high-sensitivity C-reactive protein levels in adolescents

Olaya de Dios[1] | Leticia Herrero[1] | Claudia Vales-Villamarín[1] | Ignacio Mahíllo-Fernández[2] | Leandro Soriano-Guillén[3] | Carmen Garcés[1]

[1]Lipid Research Laboratory, IIS-Fundación Jiménez Díaz UAM, Madrid, Spain

[2]Department of Biostatistics and Epidemiology, IIS-Fundación Jiménez Díaz UAM, Madrid, Spain

[3]Department of Pediatrics, IIS-Fundación Jiménez Díaz UAM, Madrid, Spain

**Correspondence**
Carmen Garcés, Lipid Research Laboratory, IIS-Fundación Jiménez Díaz, Avd. Reyes Católicos 2, 28040 Madrid, Spain.
Email: cgarces@fjd.es

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## Abstract

**Background:** The association of sex hormones with C-reactive protein (CRP) levels has been reported. However, this association remains unexplored in children in whom important anthropometric and hormonal changes are taking place.

**Objectives:** To analyze the association between high-sensitivity CRP (hs-CRP) and testosterone, estradiol and sex hormone-binding globulin (SHBG) levels in a population-based sample of adolescents, and to evaluate the influence of leptin levels on this association.

**Materials and Methods:** The sample population of this cross-sectional study was comprised of 338 male and 385 female adolescents, aged 12–16 years. Information on anthropometric variables, hormone, leptin, and hs-CRP levels was available.

**Results:** In male adolescents in our study, higher age is significantly associated with higher testosterone levels and with lower leptin and SHBG concentrations across the range of age studied. No significant changes in leptin and SHBG levels by age are observed in females. In males, leptin correlates negatively with testosterone levels (−0.263, $p < 0.001$), showing a stronger correlation after adjusting by body mass index (BMI) (−0.424, $p < 0.001$). A significant correlation between hs-CRP and testosterone levels is observed in males after adjusting by BMI, but the correlation disappears after adjusting by leptin. No association between testosterone and hs-CRP was observed in females. The negative association between hs-CRP levels and SHBG remains significant after adjusting by leptin in both sexes but disappears in males after adjusting by BMI.

**Conclusion:** The negative association between hs-CRP and testosterone concentrations observed in 12- to 16-year-old males seems to be related to leptin levels which are closely negatively related to testosterone levels in males independently of BMI.

KEYWORDS
adolescents, hs-CRP, leptin, SHBG, testosterone

## 1 | INTRODUCTION

Pathological processes related to the development of arteriosclerosis begin early in life.[1] Low-grade inflammation has been related to early atherosclerotic vessel injury in children with type 1 diabetes and apparently healthy children with obesity.[2] Increased adipose tissue in obesity is implicated in the development of this low-grade inflammatory state associated with the early stages of atherogenesis.[3,4] C-reactive protein (CRP) is an inflammatory marker that has been consistently associated with obesity and has been related to all stages of atherosclerosis.[5] Adipocytokines derived from adipose tissue, particularly leptin, stimulate hepatic inflammation, inducing the production of CRP.[6] It has been suggested that these two biomarkers are able to reciprocally regulate their bioavailability.[7] In children at two different ages, we have previously described a positive correlation between CRP and leptin levels.[8]

A role of sex steroid hormones in inflammatory processes has been stated, admitting the anti-inflammatory effect of testosterone and the proinflammatory properties of estrogen. In cross-sectional studies in adults, a negative association of CRP with testosterone levels and a positive association with estradiol levels have been reported.[9–15] The association of CRP levels with sex steroids and with the circulating glycoprotein involved in their transportation, sex hormone-binding globulin (SHBG), has also been investigated in some studies in children. Negative associations of CRP with testosterone levels in obese males[16] and positive association with estradiol[17] have been reported and its association with SHBG.[18,19] However, the studies in children are scarce and, to our knowledge, the relationship of sex steroids with CRP concentrations has not been investigated in a general population of Caucasian adolescents. Adolescence is an age at which important puberty-related anthropometric and hormonal changes are taking place. A minimum body fat seems to be necessary to trigger puberty, and an implication of the adipose-derived leptin on it and its related changes have been suggested.[20] Thus, our cross-sectional study aimed to investigate the relationship of sex hormones and SHBG with high-sensitivity CRP (hs-CRP) concentrations in a cohort of 12- to 16-year-old children and to analyze the role of leptin levels on this association.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

Subjects were part of a cross-sectional study examining cardiovascular risk factors in Spanish school children.[21] The current study comprises 723 adolescents, aged 12–16 years old, of whom we have information regarding anthropometrical data and leptin, hs-CRP, and hormone concentrations. All participant reported by their parents to be suffering from chronic diseases, including precocious and delayed puberty, under any chronic therapy and/or taking oral contraceptives were excluded. The study protocol was approved by the Ethics Committee of Clinical Investigation of the IIS-Fundación Jiménez Díaz (PIC016-2019 FJD). The investigation fulfills the principles contained in the Declaration of Helsinki and subsequent reviews and the prevailing Spanish legislation on clinical research in human subjects. Parents or legal guardians were required to give written consent for their children to participate in the study.

### 2.2 | Anthropometric variables

Measurements were taken with adolescents lightly dressed and barefoot. Weight was determined to the nearest 0.1 kg using a standardized electronic digital scale, and height was measured to the nearest 0.1 cm using a portable stadiometer. Body mass index (BMI, weight in kilograms divided by height in meters squared, $kg/m^2$) was calculated from these parameters.

### 2.3 | Biochemical data

Blood samples were obtained early in the morning after overnight fasting. Leptin concentrations were determined by enzyme-linked immunosorbent assays (ELISA) using a commercial kit (EIA-2395, DRG). CRP levels were measured using a high-sensitivity C-Reactive Protein ELISA kit (CRP High Sensitivity SK00080-02, Aviscera Bioscience, Inc.). Hs-CRP levels were measured with a detection limit of 0.15 mg/L, with intra- and inter-assay coefficients of variation of 4% and 9%, respectively. Children with hs-CRP levels greater than or equal to 10 mg/L were eliminated from the study to exclude children with acute infection.

Testosterone and estradiol were determined by radioimmunoassay (RIA) (DSL-4000 Active® Testosterone; DSL-43100 Active® Estradiol), and SHBG was measured using an immunoradiometric assay (IRMA) (DSL-7400 Active® SHBG) according to manufacturer's protocols (Diagnostic Systems Laboratories, Inc.). The theoretical sensitivities of our testosterone, estradiol, and SHBG assays are 0.28 nmol/L, 40 pmol/L, and 3 nmol/L, respectively. Free testosterone concentrations and free estradiol were calculated in all subjects from the concentrations of total testosterone, total estradiol, SHBG, and albumin by the formula Vermuelen et al[22] validated by Rinaldi et al.[23]

### 2.4 | Statistical analysis

Statistical analysis was performed using the IBM SPSS software package (Version 25.0) and GraphPad Prism statistical software (Version 8). The normality of the distribution of the variables under study was examined using the Kolmogorov–Smirnov test. The characteristics of the participants were summarized as median and interquartile range, and their comparisons between boys and girls were made using Mann-Whitney *U*-test. Differences in leptin and hormone levels by age in males and females, adjusted by

BMI, were compared by analysis of covariance (ANCOVA). Spearman's simple and partial correlation adjusted by cofounding factors were used to evaluate the associations between leptin, hs-CRP, and hormone concentrations. BMI and leptin have been considered as confounders as their contribution to CRP levels may emerge independent and additional to their relationship with hormone levels. To evaluate the contribution of leptin and hs-CRP concentrations to hormone levels, multiple linear regression analyses were performed with SHBG, testosterone, and estradiol as dependent variables and with leptin and hs-CRP as independent variables in the models. Both leptin and hs-CRP were included in the models to evaluate the relationship of hs-CRP with hormone levels with the presence of leptin in the model. Due to the important collinearity between BMI and leptin levels, BMI has been excluded from this analysis. Log-transformed variables have been used since all these variables showed a non-normal distribution. To further analyze the relationship between hs-CRP and hormones levels, we categorized hs-CRP in three groups as follows: (a) hs-CRP under the detection limit (<0.15 mg/L); (b) hs-CRP>0.15 mg/L and <75th percentile of those with hs-CRP values over the detection limit (<0.80 mg/L); (c) hs-CRP>75th sex-specific percentile of those with hs-CRP values over the detection limit (>0.80 mg/L). Testosterone and SHBG levels across hs-CRP groups, adjusted for BMI and leptin levels, were compared by analysis of covariance (ANCOVA), with Tukey's correction for multiple comparisons.

## 3 | RESULTS

The study included 338 males and 385 females. The characteristics (age, BMI, and biochemical parameters) of the study participants according to sex are shown in Table 1. Males showed significantly lower leptin levels than females. SHBG levels were significantly higher in females than in males. hs-CRP levels were not significantly different between sexes.

**TABLE 1** Characteristics (median (IQR)) of the study participants

| | Males ($n$ = 338) | Females ($n$ = 385) |
|---|---|---|
| Age (years) | 14.5 (13.6, 15.2) | 14.4 (13.7, 15.1) |
| BMI (kg/m$^2$) | 21.2 (19.4, 24.2) | 21.3 (19.6, 23.6) |
| hs-CRP (mg/L) | 0.32 (0.12, 0.88) | 0.28 (0.10, 0.62) |
| Leptin (ng/ml) | 3.58 (1.46, 6.80) | 11.5 (7.36, 17.7)* |
| SHBG (nmol/L) | 41.2 (27.1, 62.0) | 57.1 (40.8, 78.6)* |
| Total testosterone (nmol/L) | 17.5 (8.48, 22.7) | 2.64 (1.70, 4.04)* |
| Free testosterone (nmol/L) | 0.30 (0.13, 0.49) | 0.03 (0.02, 0.05)* |
| Total estradiol (pmol/L) | 125 (96.2, 160) | 225 (163, 361)* |
| Free estradiol (pmol/L) | 2.88 (2.10, 3.97) | 4.63 (3.38, 8.24)* |

*Note:* $p$-value: Mann-Whitney $U$-test; *$p$ < 0.001.

### 3.1 | Leptin and hormone concentrations by age

Leptin and hormone levels, adjusted by BMI, in males and females by age-group are shown in Table 2. Higher age was associated with significantly ($p$ < 0.001) higher testosterone levels and significantly ($p$ < 0.001) lower leptin and SHBG concentrations in male adolescents across the range of age studied. In females, no significant differences in mean leptin and SHBG concentrations between groups of age were observed. Estradiol and testosterone concentrations showed significant ($p$ < 0.01) differences across age-groups.

### 3.2 | Correlation of leptin concentrations with hs-CRP and hormone levels

Correlation analysis of leptin with hs-CRP and hormonal levels was performed adjusted by BMI since leptin had an important correlation with BMI (Table 3). After adjusting for BMI, the positive association between leptin and hs-CRP levels remained significant only in males.

The negative correlation between leptin and both total and free testosterone observed in males emerges stronger after adjusting by BMI (−0.424, $p$ < 0.001 and −0.435, $p$ < 0.001, respectively). A significant ($p$ < 0.001) positive correlation with SHBG and a negative correlation with estradiol appear in males after adjusting by BMI (Table 3). To further understand the associations that emerge when adjusting by BMI, an analysis was performed adjusting for testosterone levels that revealed a significant negative correlation between leptin and SHBG ($r$ = −0.218; $p$ < 0.001) and significant positive correlations between leptin and total estradiol and free estradiol ($r$ = 0.121, $p$ < 0.05 and $r$ = 0.167, $p$ < 0.01).

In females, the negative correlation between leptin and SHBG disappears after adjusting by BMI, while remains significant its positive correlations with sex hormone levels (Table 3).

### 3.3 | Relationship between hs-CRP and hormone levels

Table 4 shows the correlations between hs-CRP and hormone levels unadjusted and adjusted by BMI and leptin. In males, we observed a correlation between hs-CRP and testosterone that appears higher after adjusting by BMI and disappears after adjusting by leptin. The negative correlation between hs-CRP and SHBG and the positive correlations between hs-CRP and free estradiol remain significant after adjusting by leptin but disappear after adjusting by BMI (Table 4). In females, hs-CRP levels correlated only with SHBG levels. This negative correlation remains significant after adjusting by BMI and by leptin.

Table 5 shows the results of the multiple regression analyses with hormone levels as dependent variables. In male adolescents, we observed a significant negative contribution of leptin to testosterone levels. In contrast, a positive contribution of leptin to testosterone levels was observed in females. However, hs-CRP concentrations

**TABLE 2** Leptin and sexual hormones levels (mean ± *SE*) by age adjusted by BMI

| | Boys | | | | | |
|---|---|---|---|---|---|---|
| | 12 years (*n* = 29) | 13 years (*n* = 46) | 14 years (*n* = 91) | 15 years (*n* = 109) | 16 years (*n* = 63) | *p*-value |
| Leptin (ng/ml) | 9.3 ± 1.1 | 7.7 ± 0.8 | 6.5 ± 0.6 | 5.0 ± 0.5 | 3.2 ± 0.7 | <0.001 |
| SHBG (nmol/L) | 85.4 ± 5.6 | 58.5 ± 4.1 | 54.2 ± 2.9 | 40.2 ± 2.7 | 34.7 ± 3.9 | <0.001 |
| Testosterone (nmol/L) | 6.3 ± 1.7 | 10.9 ± 1.3 | 15.9 ± 0.9 | 20.5 ± 0.8 | 21.8 ± 1.2 | <0.001 |
| Estradiol (pmol/L) | 108.7 ± 14.2 | 124.5 ± 10.5 | 139.3 ± 7.4 | 136.1 ± 6.8 | 176.3 ± 9.8 | <0.01 |
| | Girls | | | | | |
| | 12 years (*n* = 28) | 13 years (*n* = 38) | 14 years (*n* = 140) | 15 years (*n* = 116) | 16 years (*n* = 61) | *p*-value |
| Leptin (ng/ml) | 12.8 ± 1.5 | 12.9 ± 1.3 | 13.7 ± 0.6 | 15.0 ± 0.7 | 15.7 ± 1.0 | NS |
| SHBG (nmol/L) | 64.1 ± 6.5 | 62.8 ± 5.4 | 68.6 ± 2.8 | 60.4 ± 3.1 | 57.0 ± 4.4 | NS |
| Testosterone (nmol/L) | 2.7 ± 0.5 | 2.6 ± 0.4 | 3.0 ± 0.2 | 3.2 ± 0.2 | 4.4 ± 0.3 | <0.01 |
| Estradiol (pmol/L) | 173.9 ± 57.0 | 315.3 ± 47.4 | 299.6 ± 24.5 | 376.0 ± 26.9 | 430.2 ± 38.7 | <0.01 |

*Note: p*-value: ANCOVA.

**TABLE 3** Correlation analysis of leptin with hs-CRP and hormone levels

| | Males (*n* = 338) | | Females (*n* = 385) | |
|---|---|---|---|---|
| | Unadjusted | BMI adjusted | Unadjusted | BMI adjusted |
| BMI | 0.581*** | | 0.665*** | |
| hs-CRP | 0.368*** | 0.130* | 0.169** | 0.012 |
| SHBG | −0.041 | 0.270*** | −0.289*** | −0.053 |
| Testosterone | −0.263*** | −0.424*** | 0.234*** | 0.135** |
| Free testosterone | −0.190*** | −0.435*** | 0.318*** | 0.139** |
| Estradiol | −0.005 | −0.103 | 0.127* | 0.126* |
| Free estradiol | 0.027 | −0.201*** | 0.229*** | 0.136** |

*Note: p*-value: significance of correlation; $p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Abbreviations: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; SHBG, sex hormone-binding globulin.

**TABLE 4** Correlation analysis between hs-CRP and hormone levels

| | Males (*n* = 338) | | | Females (*n* = 385) | | |
|---|---|---|---|---|---|---|
| | Unadjusted | BMI adjusted | Leptin adjusted | Unadjusted | BMI adjusted | Leptin adjusted |
| BMI | 0.395*** | | 0.255*** | 0.209*** | | 0.185*** |
| SHBG | −0.148** | −0.013 | −0.163** | −0.184** | −0.119* | −0.156** |
| Testosterone | −0.113* | −0.145** | −0.004 | −0.008 | −0.088 | −0.081 |
| Free testosterone | −0.029 | −0.114* | 0.053 | 0.072 | −0.013 | 0.013 |
| Estradiol | 0.094 | 0.018 | 0.086 | −0.076 | −0.075 | −0.084 |
| Free estradiol | 0.148** | 0.022 | 0.137* | −0.002 | −0.041 | −0.037 |

*Note: p*-value: significance of correlation: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Abbreviations: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; SHBG, sex hormone-binding globulin.

were not independently associated with testosterone levels when they were included in the multiple linear regression models with leptin. Leptin showed a negative significant association with SHBG levels in girls and no association in boys, with CRP levels showing weak associations with SHBG in both sexes. The results reported in girls were similar to the results of an analysis that excluded the 34 girls who had not reached menarche and to the results of the analysis in this small group of girls without menarche (data not shown).

To further investigate these relationships, testosterone and SHBG levels were analyzed by hs-CRP concentration groups: Group

**TABLE 5** Multiple linear regression analysis with SHBG, testosterone, and estradiol as dependent variables

| | Log-SHBG B (Std error) | Log-testosterone B (Std error) | Log-estradiol B (Std error) |
|---|---|---|---|
| Boys | | | |
| Log-leptin | −0.002 (0.033) | −0.208 (0.043)** | 0.008 (0.046) |
| Log-hs CRP | −0.069 (0.023)* | −0.011 (0.030) | 0.025 (0.017) |
| $R^2$ (%) | 2.8 | 7.3 | 0.9 |
| Girls | | | |
| Log-leptin | −0.201 (0.041)** | 0.194 (0.041)** | 0.136 (0.050)* |
| Log-hs CRP | −0.059 (0.020)* | −0.033 (0.019) | −0.044 (0.023) |
| $R^2$ (%) | 9.2 | 5.6 | 2.4 |

*Note:* B: regression coefficient.
Significance: *$p < 0.01$; **$p < 0.001$.

1 (hs-CRP ≤0.15 mg/L), Group 2 (0.15 mg/L <hs-CRP ≤0.80 mg/L) and group 3 (hs-CRP >0.80 mg/L) unadjusted, BMI adjusted, and leptin adjusted (Figure 1). In males, there was a significant reduction in mean testosterone levels from the lowest hs-CRP concentration group to the highest; differences in testosterone levels between hs-CRP concentration groups remain significant after adjusting by BMI but disappear after adjusting by leptin. No significant differences in testosterone concentration by hs-CRP group were found in females.

Significant differences in SHBG levels across the hs-CRP groups were observed in both males ($p < 0.05$) and females ($p < 0.01$), which was maintained adjusting by BMI and adjusting by leptin in females, but was lost after adjusting by BMI in males.

## 4 | DISCUSSION

In our study, we investigated the relationship of hs-CRP concentrations with sex hormones levels in a population-based sample of 12- to–16-year-old children, analyzing the role of leptin on this association. Our hypothesis regarding a possible role of leptin on this association was based on two facts, on the one hand, we had previously described that leptin levels are significantly related to increased hs-CRP levels after adjusting for BMI, particularly in boys[8]; on the other hand, an important inhibitory action of testosterone on leptin production has been described.[24]

In males in our study, we found a significant negative correlation between testosterone and hs-CRP levels that remains significant when adjusting by BMI, but that disappears after adjusting by leptin levels. No association between testosterone and hs-CRP levels was observed in females. Therefore, according to these findings, the role of leptin on the association between testosterone levels and hs-CRP in males can be recognized. The association between hs-CRP and testosterone has been described in other studies in children,[16,25] but, to our knowledge, the influence of leptin levels on

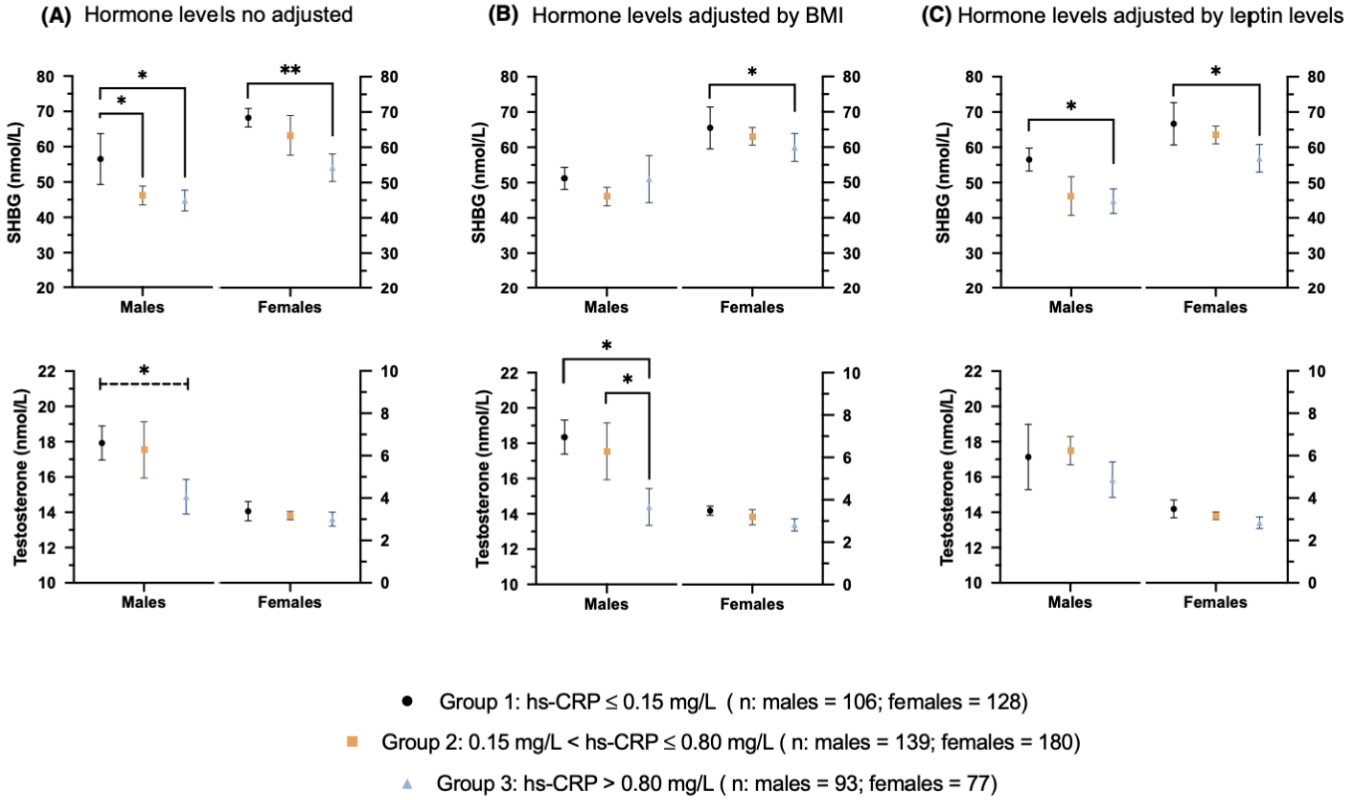


**FIGURE 1** SHBG and testosterone levels (means and 95% CI) across hs-CRP groups unadjusted (A) and adjusting by BMI (B) and by leptin levels (C). *p*-value: ANCOVA with Tukey's correction. *$p < 0.05$; **$p < 0.01$

this association has not been investigated before. As previously described in our population,[26] males in our study were in a moment of important changes, affecting both growth and hormonal levels, and reflected in a considerable increase in their body weight across the period of age studied. No such changes are occurring in females. A different growth pattern has been described in males and females. The growth rate peak takes place earlier in females than in males.[27] Therefore, the lack of changes in females in our study may be due to the fact that by the age of 12 they had already experienced their peak of growth, just before the studied period. The males in our study, on the other hand, were in full ponderal and hormonal change, as the peak of growth is taking place within the studied period.[26] As report here, in these males, after adjusting by BMI, a higher age is associated with higher testosterone levels and lower leptin. In females, however, no significant differences were observed in testosterone or leptin levels among the age-groups after adjusting by BMI. This negative inflexion in leptin levels occurring after the testosterone rises in males has been described in other cross-sectional studies in boys[28] and supports an inhibitory action of testosterone on leptin production. The lack of this association in females could be related to the very low levels of testosterone observed in females, unable to have an action on leptin production. Due to the existence of a high positive correlation between BMI and leptin levels, BMI appears as a confounding factor in assessing the association of leptin with testosterone levels, in fact the correlation between leptin and testosterone concentrations appears higher in males when adjusting by BMI.

When analyzing the association of leptin with estradiol and as SHBG in these males, we did not find any significant correlation in the unadjusted analysis, but surprisingly positive correlations with SHBG and negative correlations with estradiol appears, which reverse to negative correlations for SHBG and positive for estradiol when adjusting the analysis by testosterone, supporting the fact that testosterone, affecting leptin levels, is also affecting the association of leptin with estradiol and SHBG in males.

In females in our study, we observed a negative association of leptin levels with SHBG and positive associations with all sex hormone levels. After adjusting by BMI, the association of leptin with SHBG levels disappears, while its association with sex hormone emerges weaker. It has been described that SHBG concentrations rise in childhood, reach a plateau, and decline before puberty,[29,30] probably to enhance steroid bioavailability. It has been suggested that the increase in body mass occurring at this age could be related to this decline of SHBG levels. We have previously described a negative association of SHBG concentration with BMI and waist circumference in adolescents.[21] Adiposity-associated signals have been suggested to suppress SHBG levels.[19] Our results support the association of the leptin concentrations closely related to BMI with SHBG levels and then bioavailability of sex steroids in females, while in males, as discussed before, testosterone is controlling leptin levels and its effect on SHBG.

In our study, we found a negative relationship of SHBG with hs-CRP concentrations in males and females, although the association disappears after adjusting by BMI in males. It is known that, at the age of children in our study, body fat percentage is characteristically superior in females than in males,[31] thus the known inflammatory action of fat mass[32] might justify the consistent association between lower levels of SHBG and CRP, particularly in females.

Gender differences in CRP levels during the first decades of life have been described[33] and have been related to a gender-specific regulation of CRP. In our study, we show that gender-related differences in sex steroid hormones affect hs-CRP, thus showing their role in low-grade chronic inflammation.

As a limitation to our findings, we should mention that, unfortunately, given the cross-sectional nature of our study, it cannot be established the temporal sequence of the observed associations, further studies are needed to clarify these relationships. The lack of information on fat mass in our population and on timing of blood sampling according to menstrual cycle in girls appear as another important limitations in our study.

## 5 | CONCLUSION

Our findings show that leptin concentrations seem to be stronger negatively associated with testosterone levels in adolescent males compared with the association between hs-CRP and testosterone levels, since the negative relationship between testosterone and hs-CRP levels in males in our study disappears after adjusting for leptin.

### ACKNOWLEDGEMENTS

The article is dedicated to the late Prof. Manuel de Oya as the warmest homage to his memory.

### CONFLICT OF INTEREST

None of the authors has a conflict of interest.

### AUTHOR CONTRIBUTIONS

O.D., L.H., and C.V.-V. carried out laboratory work. O.D. and C.G. analyzed the data. L.S.-G. contributed essential resources; I.M-F supervised the statistical analysis; C.G. designed the research study and wrote the paper. All authors have read and approved the final version of the manuscript.

### ORCID

*Olaya de Dios* https://orcid.org/0000-0003-4839-0071
*Carmen Garcés* https://orcid.org/0000-0002-1099-2061

### REFERENCES

1. Tuzcu EM, Kapadia SR, Tutar E, et al. High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults.

*Circulation*. 2001;103(22):2705-2710. https://doi.org/10.1161/01.CIR.103.22.2705

2. Mangge H, Schauenstein K, Stroedter L, Griesl A, Maerz W, Borkenstein M. Low grade inflammation in juvenile obesity and type 1 diabetes associated with early signs of atherosclerosis. *Exp Clin Endocrinol Diabetes*. 2004;112(7):378-382. https://doi.org/10.1055/s-2004-821023
3. Kapiotis S, Holzer G, Schaller G, et al. A proinflammatory state is detectable in obese children and is accompanied by functional and morphological vascular changes. *Arterioscler Thromb Vasc Biol*. 2006;26(11):2541-2546. https://doi.org/10.1161/01.ATV.0000245795.08139.70
4. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res*. 2005;96(9):939-949. https://doi.org/10.1161/01.RES.0000163635.62927.34
5. Lau DCW, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atheroslcerosis. *Am J Physiol-Heart Circ Physiol*. 2005;288(5):H2031-H2041. https://doi.org/10.1152/ajpheart.01058.2004
6. Loffreda S, Yang SQ, Lin HZ, et al. Leptin regulates proinflammatory immune responses. *FASEB J*. 1998;12(1):57-65. https://doi.org/10.1096/fsb2fasebj.12.1.57
7. Hribal M, Fiorentino T, Sesti G. Role of C Reactive Protein (CRP) in leptin resistance. *Curr Pharm Des*. 2014;20(4):609-615. https://doi.org/10.2174/13816128113199990016
8. Navarro P, de Dios O, Gavela-Pérez T, Jois A, Garcés C, Soriano-Guillén L. High-sensitivity C-reactive protein and leptin levels related to body mass index changes throughout childhood. *J Pediatr*. 2016;178:178-182. https://doi.org/10.1016/j.jpeds.2016.08.020
9. Kupelian V, Chiu GR, Araujo AB, Williams RE, Clark RV, McKinlay JB. Association of sex hormones and C-reactive protein levels in men. *Clin Endocrinol (Oxf)*. 2010;72(4):527-533. https://doi.org/10.1111/j.1365-2265.2009.03713.x
10. Tsilidis KK, Rohrmann S, Mcglynn KA, et al. Association between endogenous sex steroid hormones and inflammatory biomarkers in US men. *Andrology*. 2013;1(6):919-928. https://doi.org/10.1111/j.2047-2927.2013.00129.x
11. Zhang Y, Gao Y, Tan A, et al. Endogenous sex hormones and C-reactive protein in healthy chinese men. *Clin Endocrinol (Oxf)*. 2013;78(1):60-66. https://doi.org/10.1111/j.1365-2265.2012.04359.x
12. Joffe HV, Ridker PM, Manson JE, Cook NR, Buring JE, Rexrode KM. Sex hormone-binding globulin and serum testosterone are inversely associated with C-reactive protein levels in postmenopausal women at high risk for cardiovascular disease. *Ann Epidemiol*. 2006;16(2):105-112. https://doi.org/10.1016/j.annepidem.2005.07.055
13. Kaplan SA, Johnson-Levonas AO, Lin J, Shah AK, Meehan AG. Elevated high sensitivity C-reactive protein levels in aging men with low testosterone. *Aging Male*. 2010;13(2):108-112. https://doi.org/10.3109/13685530903440424
14. Tang YJ, Lee WJ, Chen YT, Liu PH, Lee MC, Sheu WHH. Serum testosterone level and related metabolic factors in men over 70 years old. *J Endocrinol Invest*. 2007;30(6):451-458. https://doi.org/10.1007/BF03346327
15. Nakhai Pour HR, Grobbee DE, Muller M, Van Der Schouw YT. Association of endogenous sex hormone with C-reactive protein levels in middle-aged and elderly men. *Clin Endocrinol (Oxf)*. 2007;66(3):394-398. https://doi.org/10.1111/j.1365-2265.2007.02745.x
16. Mogri M, Dhindsa S, Quattrin T, Ghanim H, Dandona P. Testosterone concentrations in young pubertal and post-pubertal obese males. *Clin Endocrinol (Oxf)*. 2013;78(4):593-599. https://doi.org/10.1111/cen.12018
17. Ağırbaşlı M, Tanrıkulu A, Azizy M. Free estradiol index levels associated with high sensitivity CRP levels in male children. *Turk J Pediatr*. 2017;59(1):49-55. https://doi.org/10.24953/turkjped.2017.01.008
18. Sørensen K, Aksglaede L, Munch-Andersen T, et al. Sex hormone-binding globulin levels predict insulin sensitivity, disposition index, and cardiovascular risk during puberty. *Diabetes Care*. 2009;32(5):909-914. https://doi.org/10.2337/dc08-1618
19. Pinkney J, Streeter A, Hosking J, Mohammod M, Jeffery A, Wilkin T. Adiposity, chronic inflammation, and the prepubertal decline of sex hormone binding globulin in children: evidence for associations with the timing of puberty (earlybird 58). *J Clin Endocrinol Metab*. 2014;99(9):3224-3232. https://doi.org/10.1210/jc.2013-3902
20. Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty 1. *J Clin Endocrinol Metab*. 1997;82(4):1066-1070. https://doi.org/10.1210/jcem.82.4.3878
21. De Oya I, Schoppen S, Lasunción MA, et al. Sex hormone-binding globulin levels and metabolic syndrome and its features in adolescents. *Pediatr Diabetes*. 2010;11(3):188-194. https://doi.org/10.1111/j.1399-5448.2009.00559.x
22. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84(10):3666-3672. https://doi.org/10.1210/jcem.84.10.6079
23. Rinaldi S, Geay A, Déchaud H, et al. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. *Cancer Epidemiol Biomarkers Prev*. 2002;11(10):1065-1071
24. Wabitsch M, Blum WF, Muche R, et al. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. *J Clin Invest*. 1997;100(4):808-813. https://doi.org/10.1172/JCI119595
25. Wen X, Pekkala S, Wang R, et al. Does systemic low-grade inflammation associate with fat accumulation and distribution? A 7-year follow-up study with peripubertal girls. *J Clin Endocrinol Metab*. 2014;99(4):1411-1419. https://doi.org/10.1210/jc.2013-3267
26. Garcés C, de Oya I, Lasunción MA, López-Simón L, Cano B, de Oya M. Sex hormone-binding globulin and lipid profile in pubertal children. *Metabolism*. 2010;59(2):166-171. https://doi.org/10.1016/j.metabol.2009.06.033
27. Tanner JM, Davies PSW. Clinical longitudinal standards for height and height velocity for North American children. *J Pediatr*. 1985;107(3):317-329. https://doi.org/10.1016/S0022-3476(85)80501-1
28. Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF. Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage 1. *J Clin Endocrinol Metab*. 1997;82(9):2849-2855. https://doi.org/10.1210/jcem.82.9.4235
29. Elmlinger MW, Kühnel W, Wormstall H, Döller PC. Reference intervals for testosterone, androstenedione and SHBG levels in healthy females and males from birth until old age. *Clin Lab*. 2005;51(11-12):625-632.
30. Handelsman DJ, Sikaris K, Ly LP. Estimating age-specific trends in circulating testosterone and sex hormone-binding globulin in males and females across the lifespan. *Ann Clin Biochem*. 2015;53(3):377-384. https://doi.org/10.1177/0004563215610589
31. Gallagher D, Visser M, Sepúlveda D, Pierson RN, Harris T, Heymsfieid SB. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol*. 1996;143(3):228-239. https://doi.org/10.1093/oxfordjournals.aje.a008733

32. Wozniak SE, Gee LL, Wachtel MS, Frezza EE. Adipose tissue: the new endocrine organ? A review article. *Dig Dis Sci*. 2009;54(9):1847-1856. https://doi.org/10.1007/s10620-008-0585-3
33. Shanahan L, Copeland WE, Worthman CM, Erkanli A, Angold A, Costello EJ. Sex-differentiated changes in C-reactive protein from ages 9 to 21: the contributions of BMI and physical/sexual maturation. *Psychoneuroendocrinology*. 2013;38(10):2209-2217. https://doi.org/10.1016/j.psyneuen.2013.04.010

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## OBJETIVO 2.3. DETERMINAR POSIBLES FACTORES RELACIONADOS CON LOS NIVELES PLASMÁTICOS DE PCR.

### OBJETIVO 2.3.2. ESTUDIAR LA RELACIÓN DE LAS CONCENTRACIONES DE PCR CON LOS NIVELES DE VITAMINAS PLASMÁTICAS EN LA PRIMERA COHORTE DEL ESTUDIO 4P.

Referencia:

de Dios O, Navarro P, Ortega-Senovilla H, Herrero L, Gavela-Pérez T, Soriano-Guillen L, Lasunción MA, Garcés C. Plasma retinol levels and high-sensitivity C-reactive protein in prepubertal children. Nutrients 2018; 10. doi:10.3390/nu10091257.


#### RESUMEN

La relación entre los niveles de proteína C-reactiva (PCR) y los antioxidantes plasmáticos se ha descrito en adultos. Sin embargo, esta asociación rara vez se ha investigado en niños sanos. Así, quisimos estudiar la asociación de los niveles de PCR de alta sensibilidad (as-PCR) con las concentraciones plasmáticas de antioxidantes liposolubles en una cohorte de niños prepúberes sanos. Los niveles de as-PCR se midieron en 543 niños sanos de seis a ocho años utilizando un kit de ensayo inmunoabsorbente ligado a enzimas (ELISA). Las concentraciones plasmáticas de lípidos, apolipoproteínas y antioxidantes liposolubles (α-tocoferol, γ-tocoferol, licopeno, α-caroteno, β-caroteno y retinol) se determinaron mediante métodos estandarizados. El análisis de correlación de Pearson mostró correlaciones significativas entre las concentraciones plasmáticas de as-PCR y α-caroteno y retinol. Después de ajustar por sexo, índice de masa corporal (IMC) y niveles lipídicos, únicamente la asociación con retinol siguió siendo significativa. Además, los niños situados en el tercil con niveles más elevados de as-PCR (as-PCR ≥ 0,60 mg/dL) mostraron niveles de retinol significativamente más bajos que los que se encontraban en el primer o segundo tercil. Un análisis de regresión lineal seleccionó como predictores de los niveles de as-PCR al retinol, el IMC, apo A-I y el sexo, en un modelo que explicó el 19,2% de la variabilidad. En conclusión, en niños prepúberes sanos, tras ajustar por sexo, IMC y niveles lipídicos, las concentraciones de as-PCR se asociaron en gran medida con los niveles plasmáticos de retinol, que se transportan en la sangre unido a la proteína de unión al retinol, pero no a los antioxidantes unidos a las lipoproteínas.

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# Plasma Retinol Levels and High-Sensitivity C-Reactive Protein in Prepubertal Children

**Olaya de Dios [1], Pilar Navarro [1], Henar Ortega-Senovilla [2], Leticia Herrero [1], Teresa Gavela-Pérez [3], Leandro Soriano-Guillen [3], Miguel A. Lasunción [4] and Carmen Garcés [1,*]**

1 Lipid Laboratory, IIS-Fundación Jiménez Díaz, UAM, 28040 Madrid, Spain; olaya.dios@quironsalud.es (O.d.D.); mpilar_ns@hotmail.com (P.N.); leticia.herrerot@quironsalud.es (L.H.)
2 Faculties of Pharmacy and Medicine, Universidad San Pablo-CEU, 28925 Madrid, Spain; henar@ceu.es
3 Department of Pediatrics, IIS-Fundación Jiménez Díaz, UAM, 28040 Madrid, Spain; TGavela@quironsalud.es (T.G.-P.); LSoriano@fjd.es (L.S.-G.)
4 Servicio de Bioquímica-Investigación, Hospital Universitario Ramón y Cajal, 28034 Madrid, Spain; miguel.a.lasuncion@hrc.es
* Correspondence: cgarces@fjd.es; Tel.: +34-91-543-2880

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**Abstract:** The relationship between C-reactive protein (CRP) levels and plasma antioxidants has been established in adults. However, the association has been rarely investigated in healthy children. Thus, we examined the cross-sectional association of high-sensitivity CRP (hs-CRP) levels with fat-soluble plasma antioxidant concentrations in a cohort of healthy prepubertal children. We determined hs-CRP levels in 543 healthy six–eight-year-old children using a high-sensitivity CRP enzyme-linked immuno sorbent assay (ELISA) kit. The plasma concentrations of lipids, apolipoproteins and lipid-soluble antioxidants (α-tocopherol, γ-tocopherol, lycopene, α-carotene, β-carotene and retinol) were determined using standardized methods. Pearson correlation analysis showed significant correlations between plasma hs-CRP and α-carotene and retinol concentrations. After adjusting by sex, body mass index (BMI) and lipid levels, only the association with retinol remains significant, with children in the highest hs-CRP tertile group (hs-CRP $\geq$ 0.60 mg/dL) showing significantly lower levels of retinol than those from the tertiles 1 and 2. A stepwise linear regression selected retinol, BMI, apo A-I and sex as predictors of hs-CRP levels, in a model explaining 19.2% of the variability of hs-CRP. In conclusion, in healthy prepubertal children, after adjusting by sex, BMI and lipid levels, hs-CRP concentrations were highly associated with plasma retinol, which is transported in blood bound to retinol-binding protein but were not associated with the lipoprotein-bound antioxidants.

**Keywords:** C-reactive protein; hs-CRP concentrations; fat-soluble plasma antioxidants; plasma retinol concentrations; prepubertal children

## 1. Introduction

C-reactive protein (CRP) is an acute-phase inflammatory protein synthesized in the liver through the stimulation of interleukin-6 that has been extensively studied as a marker of the subclinical inflammation associated with obesity, metabolic syndrome, diabetes and cardiovascular disease [1].

It is known that oxidative stress has pro-inflammatory effects, and an important role in this association has been attributed to CRP [2]. Plasma antioxidants seem to decline during the "acute-phase response" in the inflammatory process that is associated with the increase of inflammatory markers, such as CRP [3]. However, the association of these biomarkers with reduced antioxidant levels in low-grade inflammation remains under study. A meta-analysis of randomized controlled

trials analyzing the effect of vitamin E supplementation suggests that supplementation with either α-tocopherol or γ-tocopherol reduces blood CRP concentrations [4]. Schwab et al. [5] suggested that this association was found only with vitamin E in combination with the intake of other antioxidants. Epidemiological studies to date analyzing the association of dietary antioxidants and serum vitamins with CRP levels in adults have produced diverse evidence. In some studies, CRP concentrations have been related to lower levels of carotenoids, vitamin E and vitamin A levels [6–12], while other investigations have failed to find an association of CRP levels with vitamin E [13] or α-tocopherol [8], for example. Race differences in the relation of vitamins A, E and β-carotene with CRP have been reported [14].

Studies analyzing the association between CRP and antioxidant levels in children are scarce and it has been mostly investigated in sick children. The association between CRP and vitamin A, for example, has been analyzed in children with an infectious disease [15], night blindness [16] or obesity [17,18]. To our knowledge, no cross-sectional studies analyzing the association of CRP with vitamin levels have been performed in healthy children in developed countries.

In our study, we examined the association of hs-CRP levels with plasma antioxidant (α-tocopherol, γ-tocopherol, lycopene, α-carotene, β-carotene and retinol) concentrations in a cohort of healthy prepubertal children in Spain.

## 2. Materials and Methods

### *2.1. Subjects*

This is a sub-study of the "Four Provinces Study", a broad cross-sectional study designed to analyze cardiovascular risk factors in Spanish schoolchildren. Our sample comprises 543 6–8-year-old children (289 girls and 254 boys). All children included in the study were free of any endocrine, metabolic, hepatic or renal disorder. Parents were required to sign a written consent form allowing their children to participate. The study protocol was approved by the Ethics Committee of Clinical Investigation of the Fundación Jiménez Díaz. The investigation fulfills the principles contained in the Declaration of Helsinki and subsequent reviews, as well as the prevailing Spanish legislation on clinical research in human subjects.

### *2.2. Data Collection*

A team consisting of one physician and several nurses was in charge of blood extractions and physical measurements.

### *2.3. Anthropometric Variables*

Measurements (weight and height) were taken with children barefoot and wearing light clothing. Weight was determined to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Body mass index (BMI) (weight in kilograms divided by height in meters squared, $kg/m^2$) was calculated from these parameters.

### *2.4. Biochemical Data*

Blood samples were obtained early in the morning after a 12-h fasting period using venipuncture. Samples were kept on ice and sent to the laboratory for analysis. Samples were centrifuged at $1500\times g$ at 4 °C for 20 min. Once centrifuged, fractions were separated and frozen at −70 °C for future analyses.

Cholesterol and triglycerides were measured enzymatically (Menarini) with a RA-1000 Autoanalyzer (Technicon, Luton, UK). High-density lipoprotein (HDL) cholesterol was measured after the precipitation of apo B-containing lipoproteins with phosphotungstic acid and Mg (Boehringer Mannheim, Baden-Wurttemberg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated according to Friedewald´s formula. Plasma apo A-I and apo B concentrations were measured using

immunonephelometry (Dade Berhing, Deerfield, IL, USA). The interassay coefficients of variation were: cholesterol, 1.4%; triglyceride, 1.7%; apo A-I, 1.55% and apo B, 4.8%.

Plasma α-tocopherol, γ-tocopherol, lycopene, α-carotene, β-carotene and retinol were measured using isocratic high-performance liquid chromatography-based methods (HPLC) (Beckman System Gold High Performance Liquid Chromatograph, NM, USA) based on the method described by H. Ortega et al. [19]. Retinol acetate and tocopherol acetate were used as internal standards. The standard reference material SRM 968c from the National Institute of Standards and Technology was used as a control. The interassay coefficients of variation were: α-tocopherol, 5.0 %; γ-tocopherol, 12.5%; lycopene, 5.5%; α-carotene, 8.8%; β-carotene, 7.8% and retinol, 4.4%.

CRP levels were measured using a high-sensitivity CRP ELISA kit (SK00080-02, Aviscera Bioscience, Inc., Santa Clara, CA, USA). The sensitivity of the assay was 0.15 mg/L.

### *2.5. Statistical Analysis*

Children with hs-CRP levels equal to or above 10 mg/L were excluded from the analysis. The normality of distributions of the variables was analyzed using the Kolmogorov-Smirnov test. Variables that were not normally distributed (hs-CRP, BMI, triglyceride, α-tocopherol, lycopene, α-carotene and β-carotene) were log-transformed to normality prior to the analyses. Mean values are shown as means ± SD. We used a t-test to compare the study variables by sex. Pearson correlation coefficients were calculated to evaluate the correlations between hs-CRP and lipid variables and fat-soluble antioxidants. To ascertain the independent predictors of plasma hs-CRP levels, a stepwise lineal regression analysis was performed. For this, the independent variables were selected among BMI, plasma lipid and apolipoprotein concentrations and fat-soluble antioxidant levels. An analysis of variance (ANOVA) was used to compare the means of the antioxidant levels between groups depending on different hs-CRP levels (hs-CRP tertiles), after adjusting for possible confounding factors (sex, age, BMI, lipid and apolipoprotein levels). Post-hoc multiple comparisons were performed using the Tukey's test. A statistical analysis was performed using the SPSS software package, version 21.0 (SPSS, Inc., Chicago, IL, USA).

## 3. Results

The average age of the children in our study was 6.7 years, similar in both genders. Mean BMI and biochemical variables by gender are shown in Table 1. No statistically significant differences in BMI, plasma lipid and apolipoprotein levels or hs-CRP concentrations between boys and girls were found (Table 1).

**Table 1.** Body mass index (BMI) and plasma biochemical variables (mean ± SD) in prepubertal children by sex.

| | **Boys (*n* = 254)** | **Girls (*n* = 287)** |
|---|---|---|
| BMI | 17.2 ± 2.6 | 17.2 ± 2.7 |
| hs-CRP (mg/L) [a] | 0.90 ± 1.52 | 0.99 ± 1.71 |
| Total cholesterol (mg/dL) | 183.9 ± 27.1 | 183.5 ± 29.1 |
| Triglycerides (mg/dL) [a] | 70.7 ± 27.9 | 72.4 ± 24.0 |
| HDL cholesterol (mg/dL) | 59.4 ± 13.1 | 58.5 ± 13.5 |
| LDL cholesterol (mg/dL) | 110.3 ± 26.6 | 110.5 ± 26.9 |
| Apo A-I (mg/dL) | 136.0 ± 18.4 | 133.6 ± 18.1 |
| Apo B (mg/dL) | 70.2 ± 14.2 | 70.8 ± 15.0 |
| α-tocopherol (mg/L) [a] | 9.07 ± 1.61 | 9.37 ± 1.74 * |
| γ-tocopherol (mg/L) | 0.95 ± 0.40 | 0.95 ± 0.41 |
| Lycopene (mg/L) [a] | 0.187 ± 0.129 | 0.183 ± 0.129 |
| α-carotene (mg/L) [a] | 0.033 ± 0.023 | 0.031 ± 0.020 |
| β-carotene (mg/L) [a] | 0.129 ± 0.074 | 0.123 ± 0.066 |
| Retinol (mg/L) | 0.29 ± 0.06 | 0.30 ± 0.064 |

[a] Log-transformed variables, * $p < 0.05$. hs-CRP: High sensitivity CRP, HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Pearson´s correlation coefficients between hs-CRP levels and BMI and plasma antioxidants, and between these vitamins and lipid and apolipoprotein concentrations are shown in Table 2. There were significant correlations between hs-CRP and BMI, β-carotene and retinol concentrations. As previously described in our population, hs-CRP also correlated negatively with HDL cholesterol and apo A-I, and positively with triglycerides (TG) concentrations [20]. As expected, plasma concentrations of fat-soluble antioxidants were significantly correlated with plasma lipids. α-tocopherol was correlated with all the lipid variables; the highest correlation coefficients being found with total cholesterol and apo B concentrations. γ-tocopherol was correlated with total cholesterol, LDL cholesterol and apo B. Lycopene and carotenes positively correlated with total cholesterol, lipoprotein-cholesterol and apolipoproteins levels, but not with triglyceride. Plasma retinol concentration showed a positive correlation with HDL cholesterol, apo A-I and apo B (Table 2).

**Table 2.** Pearson correlation coefficients between plasma concentrations of fat-soluble antioxidants and lipids, apolipoproteins, high sensitivity-CRP and BMI in children.

| | hs-CRP [a] | BMI [a] | Triglycerides [a] | Cholesterol | LDL-C | HDL-C | Apo B | Apo A-I |
|---|---|---|---|---|---|---|---|---|
| α-Tocopherol [a] | −0.013 | −0.012 | 0.134 ** | 0.421 *** | 0.296 *** | 0.250 *** | 0.459 *** | 0.256 *** |
| γ-Tocopherol | 0.033 | 0.082 | 0.047 | 0.136 ** | 0.104 * | 0.058 | 0.141 *** | 0.059 |
| Lycopene [a] | −0.073 | 0.065 | −0.064 | 0.119 ** | 0.062 | 0.149 *** | 0.085 * | 0.216 *** |
| α-Carotene [a] | −0.066 | −0.085 | −0.074 | 0.151 *** | 0.112 ** | 0.129 ** | 0.144 *** | 0.133 ** |
| β-Carotene [a] | −0.144 *** | −0.074 | −0.065 | 0.219 *** | 0.172 *** | 0.147 *** | 0.226 *** | 0.173 *** |
| Retinol | −0.280 *** | 0.192 *** | 0.063 | 0.071 | −0.030 | 0.167 *** | 0.125 ** | 0.214 *** |

[a] Log-transformed skewed data. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

For the analysis, the sample was divided into tertiles of hs-CRP levels. Children in the highest hs-CRP tertile group (hs-CRP $\geq$ 0.60 mg/dL) presented significantly lower levels of retinol and β-carotene than those from tertiles 1 and 2 (Figure 1). These differences only remain significant when adjusting for BMI and lipid levels for plasma retinol concentrations (0.270 $\pm$ 0.063 in tertile 3 versus 0.300 $\pm$ 0.065 in tertile 2 and 0.305 $\pm$ 0.056 in tertile 1, $p < 0.001$).

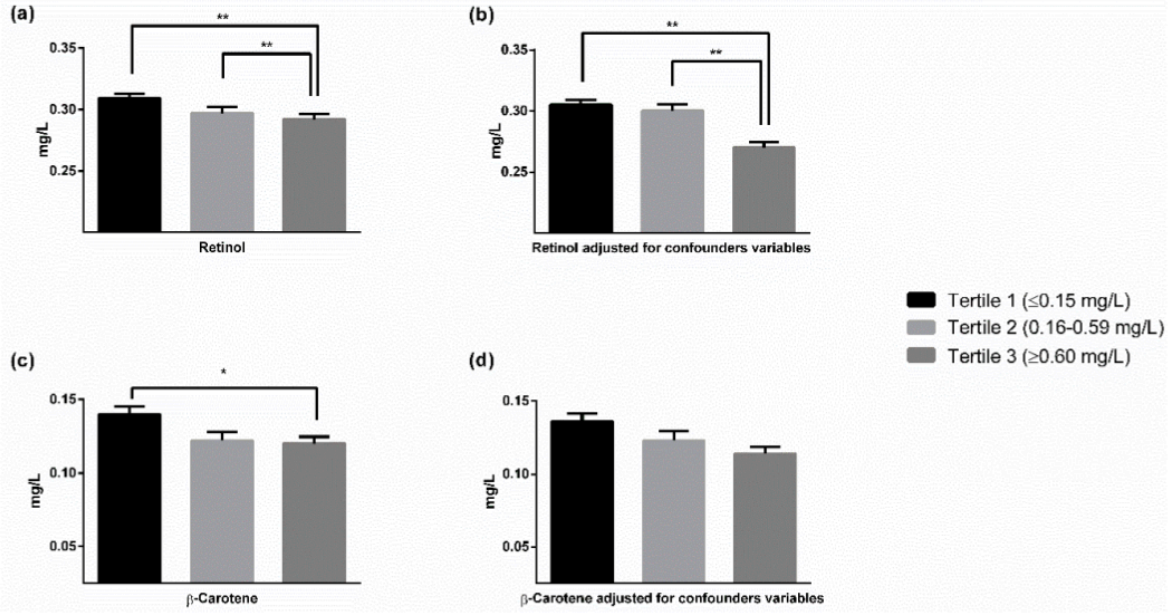


**Figure 1.** (**a**) Plasma levels of retinol (mg/L) according to hs-CRP tertiles unadjusted; (**b**) plasma levels of retinol (mg/L) according to hs-CRP tertiles adjusted for confounders variables; (**c**) plasma levels of β-Carotene (mg/L) according to hs-CRP tertiles unadjusted; (**d**) plasma levels of β-Carotene (mg/L) according to hs-CRP tertiles adjusted for confounder variables. * $p < 0.01$, ** $p < 0.001$.

We further analyzed the independent determinants of hs-CRP plasma concentrations using a stepwise linear regression. The selected variables along with the standardized coefficients (**β**) and the

p values for each predictor, and the $R^2$ values of the model are given in Table 3. Retinol, BMI, apo A-I and sex were selected as predictors in a model explaining as much as 19.2 % of the variability of hs-CRP. The magnitude of the contribution of both retinol and BMI was higher than the contribution of sex or apo A-I (Table 3).

**Table 3.** Results of linear regression model to identify independent determinants of hs-CRP plasma concentrations.

| Base Line Variable | β | *p*-Value |
|---|---|---|
| Retinol | −0.315 | <0.001 |
| BMI * | 0.287 | <0.001 |
| Apo A-I | −0.138 | 0.002 |
| Gender | 0.113 | 0.009 |

$R^2$: 19.2; * Log-transformed data.

## 4. Discussion

This study describes the relationship between the plasma concentration of fat-soluble antioxidants and hs-CRP levels in a healthy cohort of prepubertal children where the effect of sex hormones, alcohol consumption and the smoking habit can be avoided. We have identified a strong association between plasma retinol levels and hs-CRP concentrations in these children, independently of BMI and lipid levels, with our results showing significantly lower plasma retinol levels in children in the highest hs-CRP concentration tertile.

There are few published reports on the analysis of the relationship between plasma fat-soluble antioxidants and hs-CRP concentrations in prepuberal children in developed countries. Studies analyzing this association in children have focused mainly on sick children [15,16,21], and, to our knowledge, this association has not been previously described in healthy children in our area. Thus, our study adds to the literature that has investigated this association mostly in adults, showing a clear association between hs-CRP and plasma retinol levels in healthy children in a Mediterranean country, such as Spain.

In our population, even though β-carotene concentrations also appear to be related to hs-CRP levels, after adjusting by BMI and lipid levels, retinol concentration emerges as the only fat-soluble antioxidant related to hs-CRP levels in our children. Vitamin A deficiency has been associated with CRP levels among preschool children with night blindness [16] and low concentrations of vitamin A has been described in overweight and obese Mexican children [22] but, to our knowledge, no cross-sectional studies have investigated, in healthy Caucasian children, the association of hs-CRP with retinol or any of the other lipid-soluble antioxidants we have analyzed.

Interestingly, we found an association between hs-CRP levels and the plasma concentration of retinol, which is transported in blood bound to retinol-binding protein, not to lipoproteins, and we failed to find any association of hs-CRP levels with the concentrations of any of the lipoprotein-bound fat-soluble antioxidants analyzed. Since retinol concentration is tightly regulated by the retinol-binding protein (RBP) [22], we hypothesized that RBP could have a role in the association between retinol and CRP levels observed in children. A correlation between RBP4 and CRP levels has been described in obese children [23]. Further studies are needed to clarify this hypothesis.

The complex interrelatedness between CRP and oxidized low-density lipoproteins has been reviewed elsewhere [24]. The lack of an association between hs-CRP levels and the lipoprotein-bound fat-soluble antioxidant concentrations in children, which has been extensively reported in adults [6,7,9,12], may be due to the fact that the inflammatory status related to LDL oxidation is evident in adults but not in children. Thus, lipoprotein-bound antioxidants may play a role regarding LDL oxidation susceptibility in adults but not in children. The association between vitamin A and lower CRP levels has also been reported in the study by Garcia et al. [22] analyzing Mexican school-aged children. Similar to our findings for β-carotene, in this study the association between CRP

levels and vitamin E disappears when considering the vitamin E/lipids ratio [22]. Further studies are required to clarify these aspects.

In our population, we have previously described a significant association between a greater fruit and vegetable intake and lower hs-CRP levels [25], which we linked to the high fiber and antioxidant contents related to this vegetable and fruit intake. In addition to the consistent association between hs-CRP concentrations and fiber intake [10,26], the contribution of vitamin intake associated with vegetable and fruit consumption has also been accepted. A relationship between plasma hs-CRP concentration and vitamin A and vitamin E intake has been reported in our children [25]. Here, we confirmed the association between hs-CRP and retinol plasma concentrations but failed to demonstrate its association with $\alpha$-tocopherol or $\gamma$-tocopherol.

As previously described in our children, we did not find any statistically significant correlation between plasma fat-soluble antioxidants and the major nutrients or vitamins consumed [20]. As regards retinol, other studies fail to find any association between dietary intake and plasma concentration in well-nourished populations [27]. Regarding the influence of diet on serum tocopherol, studies in adult populations have provided conflicting results. Some authors have reported a small association of dietary intake of vitamin E with serum tocopherol concentration [28], while others found no significant association [27,29,30].

## 5. Conclusions

Our study in six–eight-year-old children reports an important association between hs-CRP concentrations and plasma retinol which is transported in blood bound to retinol-binding protein, and fails to find any association between hs-CRP levels and the lipoprotein-bound fat-soluble antioxidants. These findings suggest an important role of retinol in preventing inflammation in early age stages.

**Author Contributions:** H.O.-S., M.A.L. and C.G. designed the research; O.d.D., P.N., L.H. and T.G.-P. conducted the research; L.S.-G. provided the essential reagents; C.G. analyzed the data, wrote the paper and had primary responsibility for the final content. All the authors have reviewed the final version of the manuscript.

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## References

1. Kaptoge, S.; Di Angelantonio, E.; Lowe, G.; Pepys, M.B.; Thompson, S.G.; Collins, R.; Danesh, J.; Tipping, R.W.; Ford, C.E.; Pressel, S.L.; et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: An individual participant meta-analysis. *Lancet* **2010**, *375*, 132–140. [PubMed]
2. Kobayashi, S.; Inoue, N.; Ohashi, Y.; Terashima, M.; Matsui, K.; Mori, T.; Fujita, H.; Awano, K.; Kobayashi, K.; Azumi, H.; et al. Interaction of oxidative stress and inflammatory response in coronary plaque instability: Important role of C-reactive protein. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 1398–1404. [CrossRef] [PubMed]
3. Louw, J.A.; Werbeck, A.; Louw, M.E.J.; Kotze, T.J.V.W.; Cooper, R.; Labadarios, D. Blood vitamin concentrations during the acute-phase response. *Crit. Care Med.* **1992**, *20*, 934–941. [CrossRef] [PubMed]
4. Saboori, S.; Shab-Bidar, S.; Speakman, J.R.; Yousefi Rad, E.; Djafarian, K. Effect of Vitamin E supplementation on serum C-reactive protein level: A meta-analysis of randomized controlled trials. *Eur. J. Clin. Nutr.* **2015**, *69*, 867–873. [CrossRef] [PubMed]
5. Schwab, S.; Zierer, A.; Schneider, A.; Heier, M.; Koenig, W.; Kastenmüller, G.; Waldenberger, M.; Peters, A.; Thorand, B. Vitamin e supplementation is associated with lower levels of C-reactive protein only in higher dosages and combined with other antioxidants: The Cooperative Health Research in the Region of Augsburg (KORA) F4 study. *Br. J. Nutr.* **2015**, *113*, 1782–1791. [CrossRef] [PubMed]

6. Erlinger, T.P.; Guallar, E.; Miller, E.R.; Stolzenberg-Solomon, R.; Appel, L.J. Relationship between systemic markers of inflammation and serum beta-carotene levels. *Arch. Intern. Med.* **2001**, *161*, 1903–1908. [CrossRef] [PubMed]
7. Kritchevsky, S.B.; Bush, A.J.; Pahor, M.; Gross, M.D. Serum carotenoids and markers of inflammation in nonsmokers. *Am. J. Epidemiol.* **2000**, *152*, 1065–1071. [CrossRef] [PubMed]
8. Il'Yasova, D.; Ivanova, A.; Morrow, J.D.; Cesari, M.; Pahor, M. Correlation between two markers of inflammation, serum C-reactive protein and interleukin 6, and indices of oxidative stress in patients with high risk of cardiovascular disease. *Biomarkers* **2008**, *13*, 41–51. [CrossRef] [PubMed]
9. Wood, A.D.; Strachan, A.A.; Thies, F.; Aucott, L.S.; Reid, D.M.; Hardcastle, A.C.; Mavroeidi, A.; Simpson, W.G.; Duthie, G.G.; Macdonald, H.M. Patterns of dietary intake and serum carotenoid and tocopherol status are associated with biomarkers of chronic low-grade systemic inflammation and cardiovascular risk. *Br. J. Nutr.* **2014**, *112*, 1341–1352. [CrossRef] [PubMed]
10. Oliveira, A.; Rodríguez-Artalejo, F.; Lopes, C. The association of fruits, vegetables, antioxidant vitamins and fibre intake with high-sensitivity C-reactive protein: Sex and body mass index interactions. *Eur. J. Clin. Nutr.* **2009**, *63*, 1345–1352. [CrossRef] [PubMed]
11. Yang, M.; Chung, S.-J.; Floegel, A.; Song, W.O.; Koo, S.I.; Chun, O.K. Dietary antioxidant capacity is associated with improved serum antioxidant status and decreased serum C-reactive protein and plasma homocysteine concentrations. *Eur. J. Nutr.* **2013**, *52*, 1901–1911. [CrossRef] [PubMed]
12. Ford, E.S.; Liu, S.; Mannino, D.M.; Giles, W.H.; Smith, S.J. C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. *Eur. J. Clin. Nutr.* **2003**, *57*, 1157–1163. [CrossRef] [PubMed]
13. Cheng, H.G.; Alshaarawy, O.; Cantave, M.D.; Anthony, J.C. Inverse association linking serum levels of potential antioxidant vitamins with C-reactive protein levels using a novel analytical approach. *Br. J. Nutr.* **2016**, *116*, 1256–1264. [CrossRef] [PubMed]
14. Suarez, E.C.; Schramm-Sapyta, N.L. Race differences in the relation of vitamins A, C, E, and β-carotene to metabolic and inflammatory biomarkers. *Nutr. Res.* **2014**, *34*, 1–10. [CrossRef] [PubMed]
15. Mitra, A.K.; Alvarez, J.O.; Wahed, M.A.; Fuchs, G.J.; Stephensen, C.B. Predictors of serum retinol in children with shigellosis. *Am. J. Clin. Nutr.* **1998**, *68*, 1088–1094. [CrossRef] [PubMed]
16. Maqsood, M.; Dancheck, B.; Gamble, M.V.; Palafox, N.A.; Ricks, M.O.; Briand, K.; Semba, R.D. Vitamin A deficiency and inflammatory markers among preschool children in the Republic of the Marshall Islands. *Nutr. J.* **2004**, *3*, 21. [CrossRef] [PubMed]
17. Stenzel, A.P.; Carvalho, R.; Jesus, P.; Bull, A.; Pereira, S.; Saboya, C.; Ramalho, A. Serum antioxidant associations with metabolic characteristics in metabolically healthy and unhealthy adolescents with severe obesity: An observational study. *Nutrients* **2018**, *10*, 150. [CrossRef] [PubMed]
18. Yang, C.; Chen, J.; Liu, Z.; Yun, C.; Li, Y.; Piao, J.; Yang, X. Association of vitamin a status with overnutrition in children and adolescents. *Int. J. Environ. Res. Public Health* **2015**, *12*, 15531–15539. [CrossRef] [PubMed]
19. Ortega, H.; Coperías, J.L.; Castilla, P.; Gómez-Coronado, D.; Lasunción, M.A. Liquid chromatographic method for the simultaneous determination of different lipid-soluble antioxidants in human plasma and low-density lipoproteins. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2004**, *803*, 249–255. [CrossRef] [PubMed]
20. Ortega, H.; Castilla, P.; Gómez-Coronado, D.; Garcés, C.; Benavente, M.; Rodríguez-Artalejo, F.; De Oya, M.; Lasunción, M.A. Influence of apolipoprotein E genotype on fat-soluble plasma antioxidants in Spanish children. *Am. J. Clin. Nutr.* **2005**, *81*, 624–632. [CrossRef] [PubMed]
21. Beisel, W.R. Infection-induced depression of serum retinol—A component of the acute phase response or a consequence? *Am. J. Clin. Nutr.* **1998**, *68*, 993–994. [CrossRef] [PubMed]
22. García, O.P.; Ronquillo, D.; del Carmen Caamaño, M.; Martínez, G.; Camacho, M.; López, V.; Rosado, J.L. Zinc, iron and vitamins A, C and E are associated with obesity, inflammation, lipid profile and insulin resistance in Mexican school-aged children. *Nutrients* **2013**, *5*, 5012–5030. [CrossRef] [PubMed]
23. Balagopal, P.; Graham, T.E.; Kahn, B.B.; Altomare, A.; Funanage, V.; George, D. Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: Association with subclinical inflammation. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 1971–1974. [CrossRef] [PubMed]

24. Obradovic, M.M.; Trpkovic, A.; Bajic, V.; Soskic, S.; Jovanovic, A.; Stanimirovic, J.; Panic, M.; Isenovic, E.R. Interrelatedness between C-reactive protein and oxidized low-density lipoprotein. *Clin. Chem. Lab. Med.* **2015**, *53*, 29–34. [CrossRef] [PubMed]
25. Navarro, P.; de Dios, O.; Jois, A.; Gavela-Pérez, T.; Gorgojo, L.; Martín-Moreno, J.M.; Soriano-Guillen, L.; Garcés, C. Vegetable and fruit intakes are associated with hs-CRP levels in pre-pubertal girls. *Nutrients* **2017**, *9*, 224. [CrossRef] [PubMed]
26. King, D.E.; Egan, B.M.; Woolson, R.F.; Mainous, A.G.; Al-Solaiman, Y.; Jesri, A. Effect of a high-fiber diet vs a fiber-supplemented diet on C-reactive protein level. *Arch. Intern. Med.* **2007**, *167*, 502–506. [CrossRef] [PubMed]
27. Hebert, J.R.; Hurley, T.G.; Hsieh, J.; Rogers, E.; Stoddard, A.M.; Sorensen, G.; Nicolosi, R.J. Determinants of plasma vitamins and lipids: The working well study. *Am. J. Epidemiol.* **1994**, *140*, 132–147. [CrossRef] [PubMed]
28. White, E.; Kristal, A.R.; Shikany, J.M.; Wilson, A.C.; Chen, C.; Mares-Perlman, J.A.; Masaki, K.H.; Caan, B.J. Correlates of serum α- and γ-tocopherol in the women's health initiative. *Ann. Epidemiol.* **2001**, *11*, 136–144. [CrossRef]
29. Ascherio, A.; Stampfer, M.J.; Colditz, G.A.; Rimm, E.B.; Litin, L.; Willett, W.C. Correlations of Vitamin A and E Intakes with the Plasma Concentrations of Carotenoids and Tocopherols among American Men and Women. *J. Nutr.* **1992**, *122*, 1792–1801. [CrossRef] [PubMed]
30. Sinha, R.; Patterson, B.H.; Mangels, A.R.; Levander, O.A.; Gibson, T.; Taylor, P.R.; Block, G. Determinants of plasma vitamin E in healthy males. *Cancer Epidemiol. Biomarkers Prev.* **1993**, *2*, 473–479. [PubMed]


La obesidad implica un desequilibrio de la homeostasis de la energía que se asocia a una inflamación crónica de bajo grado y al desarrollo de diversas patologías. Con los trabajos incluidos en esta tesis doctoral hemos profundizado en la investigación de los péptidos implicados en la etiopatogenia de la obesidad, así como en el estado de inflamación crónica que se asocia al exceso de peso ya desde edades tempranas.

## HOMEOSTASIS DE LA ENERGÍA

Con nuestros estudios hemos querido ahondar en la implicación en la homeostasis de la energía de péptidos clásicos de adiposidad, como la leptina, e investigar el papel de "nuevos" péptidos como la nesfatina-1 y la adropina que han adquirido una especial relevancia en los últimos años.

La asociación entre los niveles de leptina e IMC, así como con presencia de obesidad, es un hito bien establecido en la literatura científica que nuestro propio grupo también ha demostrado previamente[225,226]. Sin embargo, aunque hay numerosas evidencias en modelos animales que confirman la función anorexigénica de la leptina, los resultados en estudios poblacionales a este respecto son contradictorios y escasos. Así, nuestro grupo investigó la relación entre leptina e ingesta alimentaria, sin encontrar ninguna asociación significativa. No obstante, en los últimos años, las evidencias científicas sugieren una acción conjunta de la leptina y la insulina en la regulación de la homeostasis energética en el cerebro, por lo que investigamos si una interacción entre leptina e insulina podría contribuir a explicar la regulación de la ingesta energética en nuestra población pediátrica. Teniendo en cuenta esta premisa, demostramos que la acción anorexigénica de la leptina sólo estaba presente en niñas que tenían niveles bajos de insulina, desapareciendo cuando las concentraciones de insulina eran más elevadas. Estos resultados refuerzan la idea de la existencia de funciones superpuestas de las señales de leptina e insulina regulando la ingesta. En niños, no observamos el efecto anorexigénico de la leptina, de manera dependiente o independiente de la insulina. Teniendo en cuenta el importante dimorfismo sexual que presenta la leptina ya desde etapas prepuberales y la influencia que han demostrado tener las hormonas sexuales sobre las concentraciones de leptina, podemos hipotetizar que los factores hormonales podrían tener un papel relevante en la acción de la leptina regulando la homeostasis energética.

Profundizando en el estudio de los péptidos implicados en la regulación de la homeostasis de la energía, mediante estudios caso-control, investigamos el papel de nesfatina-1 y adropina como péptidos que podrían ser clave en la etiopatogenia de la obesidad en las dos cohortes de niños integrantes del estudio 4P.

Así, nuestro estudio ha sido el primero que ha analizado la asociación entre nesfatina-1 y obesidad en edad pediátrica en función del sexo. Tanto en niñas prepuberales como en adolescentes, observamos que el grupo de niñas con obesidad presentaba niveles plasmáticos de nesfatina-1 significativamente más bajos que el grupo con normopeso. En cambio, no encontramos diferencias en los niveles de nesfatina-1 en los niños en ninguna de las dos cohortes del estudio, lo que sugiere una regulación central de la nesfatina-1 dependiente del sexo. Además, en nuestro estudio hemos observado que cuando ajustamos por el IMC se evidencia una asociación positiva de los niveles de nesfatina-1 con leptina e insulina, lo que respaldaría el papel anorexigénico de la nesfatina-1 a nivel central, pudiendo haber una sinergia entre estos péptidos anorexigénicos.

En cuanto a la adropina pudimos demostrar una asociación diferente entre sus niveles y la presencia de obesidad dependiendo de la edad y del sexo, encontrando, además, una disminución de sus concentraciones plasmáticas con la edad, particularmente en las niñas. En la edad prepuberal, observamos que tanto los niños como las niñas con obesidad presentaban concentraciones significativamente más elevadas de adropina, mientras que en edad puberal esta asociación desparecía. Otro hallazgo relevante de nuestro estudio fue la existencia de una asociación positiva entre los niveles plasmáticos de adropina y leptina tanto en niños como en niñas prepuberales, que, en edad puberal, desaparece en niños y presenta una asociación negativa en niñas. Estos resultados sugieren que la leptina junto con otras hormonas claves en la regulación del desarrollo puberal podrían contribuir a la regulación de la secreción de adropina en el sistema nervioso central.

De nuestros estudios podemos deducir que en la fisiopatología de la obesidad hay un importante dimorfismo sexual que debe de tenerse en cuenta ya en la edad pediátrica. Además, la leptina parece tener un importante papel en la homeostasis de la energía, no sólo como péptido anorexigénico sino asociado a la acción central de otros péptidos como la nesfatina-1 o la adropina.

## INFLAMACIÓN CRÓNICA Y FACTORES RELACIONADOS CON LOS NIVELES PLASMÁTICOS DE PROTEÍNA C-REACTIVA

En los últimos años, una de las líneas de investigación de nuestro grupo se ha centrado en la investigación de PCR como marcador de inflamación crónica asociado a la obesidad[177] y en el estudio de diferentes factores, como la dieta[206,207] y los polimorfismos genéticos[227], que pueden modular sus niveles plasmáticos. En todos los estudios sobre PCR, los niveles plasmáticos fueron medidos con ensayos comerciales de alta sensibilidad (as-PCR).

Respecto a la dieta, demostramos que un patrón dietético basado en el consumo de frutas y verduras se vinculaba a niveles más bajos de PCR tanto en niños de edad prepuberal como en adultos[206,207]. En la cohorte de niños prepuberales observamos, además, que el contenido dietético en vitamina A y E se relacionaba con niveles más bajos de PCR[207]. Así, decidimos investigar la asociación de los niveles plasmáticos de antioxidantes liposolubles (retinol, carotenos, tocoferoles y licopeno) con los niveles de PCR en la misma cohorte de niños prepuberales sanos. Los resultados de nuestra investigación mostraron inicialmente una asociación negativa de los niveles de PCR tanto con los niveles de retinol como de ß-caroteno, que tras ajustar por el IMC únicamente se mantuvo significativa en el caso del retinol.

Por otra parte, en nuestro estudio habíamos confirmado la validez de PCR como marcador de inflamación asociado a la obesidad[177]. Además, habíamos descrito una asociación de los niveles de PCR con los niveles de leptina independiente de IMC[177]. Esta relación leptina-PCR fue confirmada también a nivel genético al encontrar que no sólo ciertos polimorfismos de *PCR* se asociaban con niveles plasmáticos de PCR sino que también lo hacían polimorfismos de los genes *LEP* y *LEPR*[227].

Teniendo en cuenta estos resultados, quisimos ahondar en el estudio de PCR como marcador de alteraciones metabólicas englobadas en el SMet de manera independiente a las adipoquinas. Para ello tuvimos en cuenta los niveles plasmáticos de leptina y adiponectina, cuyo ratio se había relacionado con la pérdida de funcionalidad del tejido adiposo e infamación crónica, además de asociarse directamente con los propios niveles de PCR. En el estudio llevado a cabo en nuestra población adolescente, confirmamos que el ratio leptina-adiponectina es un importante biomarcador de SMet en ambos sexos; sin embargo, la validez de PCR como marcador independiente del ratio leptina-adiponectina sólo fue evidente en niñas.

Con esta investigación evidenciamos de nuevo el dimorfismo sexual que existe en torno al metabolismo y a los procesos inflamatorios asociados a su alteración, así como el posible papel de la leptina en este diferente comportamiento. Por esta razón quisimos estudiar, en esta misma

población adolescente, la relación de las concentraciones de PCR con los niveles de las hormonas sexuales teniendo en cuenta el papel de la leptina.

Teniendo en cuenta estas premisas nuestro estudio arrojó importantes resultados. En primer lugar, constatamos el papel regulador de las hormonas sexuales sobre los niveles plasmáticos de leptina, independientemente del IMC, observando una asociación que presenta signos opuestos según el sexo. Así, en niñas tanto los estrógenos como los andrógenos mostraron una asociación positiva con los niveles de leptina; en niños, por el contrario, se observó una importante asociación negativa entre testosterona y leptina. Estos resultados avalan la hipótesis de que los factores hormonales podrían tener un papel relevante en las acciones de la leptina regulando la homeostasis energética.

En cuanto a la relación entre PCR con las hormonas sexuales, en las niñas encontramos una asociación negativa entre los niveles de SHBG y PCR que se mantuvo tras el ajuste por IMC y leptina. Por el contrario, no encontramos ninguna asociación entre los niveles de PCR y estradiol o testosterona. En niños también constatamos una asociación negativa entre los niveles de SHBG y PCR, que sin embargo despareció al ajustar por el IMC. Al estudiar su relación con testosterona, en los niños sí observamos una asociación negativa entre los niveles plasmáticos de testosterona y PCR, que desapareció después de ajustar por valores de leptina, pero no tras ajustar por el IMC. Esto parece indicar que la leptina es el vínculo entre los niveles de testosterona y de PCR en varones adolescentes.

Por último, uno de los objetivos iniciales en esta tesis era profundizar en el estudio de la PCR en propio tejido adiposo (TA) para entender mejor su papel dentro de la fisiopatología de la obesidad. Así, quisimos ver si el TA visceral era una fuente extrahepática de PCR para confirmar que el TA se encontraba involucrado en la propia respuesta inflamatoria. Con nuestro estudio, en niños de edad pediátrica sometidos a una apendicetomía, demostramos la expresión proteica de PCR en el TA visceral. Además, también evidenciamos diferencias significativas en la expresión de PCR dependiendo de la gravedad de la inflamación, por lo que la expresión de PCR en TAv podría estar relacionada con el grado de inflamación local que probablemente desencadena la respuesta inflamatoria. Por lo que con nuestro estudio no sólo confirmamos que el TAv es una fuente extrahepática de PCR, sino que también podría participar activamente en la respuesta inflamatoria aumentando la producción de PCR.

Siguiendo esta línea de investigación, durante el último año hemos podido acceder a muestras de TA visceral y TA subcutáneo de adultos con obesidad mórbida sometidos a cirugía bariátrica y nos encontramos actualmente estudiando la expresión de *leptina, adiponectina y*

*PCR e IL-6* en ambos tejidos. Nuestra intención es tratar de esclarecer el papel de estas moléculas como marcadores de alteración de la funcionalidad del TA visceral y subcutáneo y sus posibles implicaciones en las complicaciones metabólicas asociadas a este tipo de patología.

1. En niñas prepuberales, la capacidad anorexigénica de la leptina parece depender de las concentraciones de insulina.
2. La nesfatina-1 parece ser un péptido anorexigénico de secreción central cuyos niveles se asocian con la presencia de obesidad únicamente en niñas. Por tanto, su mecanismo de acción parece estar regulado por el sexo.
3. Los cambios observados en los valores de adropina, así como su diferente relación con obesidad y leptina en función de la edad y el sexo apuntan a la existencia de una regulación central de su secreción influida en parte por hormonas sexuales.
4. El tejido adiposo visceral es una fuente extrahepática de PCR en edad pediátrica, estando su expresión potencialmente asociada con la gravedad de la inflamación local, lo que sugiere una participación del tejido adiposo en la repuesta inflamatoria.
5. El ratio leptina-adiponectina es un buen biomarcador de síndrome metabólico en adolescentes de ambos sexos, mientras que los valores de PCR lo son únicamente en niñas.
6. La relación de la testosterona con la inflamación, en niños puberales, estaría mediada por la acción de leptina.
7. El retinol, a diferencia de otros antioxidantes liposolubles, presenta una importante asociación negativa con los niveles de PCR. Por tanto, resultaría aconsejable el consumo de alimentos con alto contenido en retinol como estrategia para prevenir o tratar de revertir el estado inflamatorio asociado a obesidad infanto-juvenil.

1 Obesity. World Heal. Organ. https://www.who.int/health-topics/obesity#tab=tab_1.

2 Bray GA, Kim KK, Wilding JPH. Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation. *Obes Rev* 2017; **18**: 715–723.

3 WHO/NUT/NCD. OBESITY: PREVENTING AND MANAGING THE GLOBAL EPIDEMIC. Geneva, Switzerland., 2000.

4 Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: International survey. *Br Med J* 2000; **320**: 1240–1243.

5 Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012; **7**: 284–294.

6 Obesity Classification | World Obesity Federation. https://www.worldobesity.org/about/about-obesity/obesity-classification.

7 Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P *et al.* Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. *Nat Rev Endocrinol* 2020; **16**: 177–189.

8 Chooi YC, Ding C, Magkos F. The epidemiology of obesity. *Metabolism* 2019; **92**: 6–10.

9 Zimmet P, Alberti GKMM, Kaufman F, Tajima N, Silink M, Arslanian S *et al.* The metabolic syndrome in children and adolescents - An IDF consensus report. Pediatr. Diabetes. 2007; **8**: 299–306.

10 Fernández C, Lorenzo H, Vrotsou K, Aresti U, I R, Sánchez E. Estudio de Crecimiento de Bilbao. Curvas y tablas de crecimiento. Estudio Transversal. *Fund Faustino Orbegozo* 2011.

11 Ghanemi A, Yoshioka M, St-Amand J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J Clin Med* 2018; **7**: 453.

12 WHO. Obesity and overweight. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

13 Khan S, de Munter J. Statistics on Causes of Death 2019. 2020; **1**: 2019–2021.

14 Simmonds M, Llewellyn A, Owen CG, Woolacott N. Predicting adult obesity from childhood obesity: A systematic review and meta-analysis. *Obes Rev* 2016; **17**: 95–107.

15 Bentham J, Di Cesare M, Bilano V, Bixby H, Zhou B, Stevens GA *et al.* Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet* 2017; **390**: 2627–2642.

16 World Health Organization. Taking action on childhood obesity report. *World Heal Organ* 2018.

17 World Health Organization. Childhood Obesity Surveillance Initiative. 2018 doi:10.1111/j.2047-6310.2012.00068.x.

18 Foundation G. Resultados principales del estudio PASOS 2019 sobre la actividad física, los estilos de vida y la obesidad de la población española de 8 a 16 años. 2019.

19 Lloyd LJ, Langley-Evans SC, McMullen S. Childhood obesity and adult cardiovascular disease risk: A systematic review. Int. J. Obes. 2010; **34**: 18–28.

20 Maffeis C, Silvagni D, Bonadonna R, Grezzani A, Banzato C, Tatò L. Fat Cell Size, Insulin Sensitivity, and Inflammation in Obese Children. *J Pediatr* 2007; **151**: 647–652.

21 Del Villar-Rubin S, Escorihuela Esteban R, Garcia-Anguita A, Ortega Moreno L, Garcés Segura C. Valoración de la evolución temporal del sobrepeso desde la edad prepuberal hasta la adolescencia. *An Pediatr* 2013; **78**: 389–392.

22 Cunningham SA, Kramer MR, Venkat Narayan KM. Incidence of childhood obesity in the United States. *N Engl J Med* 2014; **370**: 403–411.

23 Reilly JJ, Kelly J. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: Systematic review. Int. J. Obes. 2011; **35**: 891–898.

24 Garcés C, de Oya M. Cardiovascular Risk Factors in Children. Main Findings of the Four Provinces Study. *Rev Española Cardiol (English Ed* 2007; **60**: 517–524.

25 Le Thuc O, Stobbe K, Cansell C, Nahon JL, Blondeau N, Rovère C. Hypothalamic inflammation and energy balance disruptions: Spotlight on chemokines. Front. Endocrinol. (Lausanne). 2017; **8**: 1.

26 Exley MA, Hand L, O'Shea D, Lynch L. Interplay between the immune system and adipose tissue in obesity. J.

Endocrinol. 2014; **223**: R41–R48.

27 Woods SC. The Control of Food Intake: Behavioral versus Molecular Perspectives. Cell Metab. 2009; **9**: 489–498.

28 Roh E, Song DK, Kim MS. Emerging role of the brain in the homeostatic regulation of energy and glucose metabolism. *Exp Mol Med* 2016; **48:** e216.

29 Morton GJ, Schwartz MW. Leptin and the central nervous system control of glucose metabolism. *Physiol Rev* 2011; **91**: 389–411.

30 Loh K, Zhang L, Brandon A, Wang Q, Begg D, Qi Y *et al.* Insulin controls food intake and energy balance via NPY neurons. *Mol Metab* 2017; **6**: 574–584.

31 Oh-I S, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K *et al.* Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature* 2006; **443**: 709–712.

32 Schalla MA, Stengel A. Current Understanding of the Role of Nesfatin-1. *J Endocr Soc* 2018; **2**: 1188–1206.

33 Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA, Chouljenko VN *et al.* Identification of Adropin as a Secreted Factor Linking Dietary Macronutrient Intake with Energy Homeostasis and Lipid Metabolism. *Cell Metab* 2008; **8**: 468–481.

34 Jasaszwili M, Billert M, Strowski MZ, Nowak KW, Skrzypski M. Adropin as a fat-burning hormone with multiple functions—review of a decade of research. Molecules. 2020; **25**(3):549.

35 Begg DP, Woods SC. The endocrinology of food intake. Nat. Rev. Endocrinol. 2013; **9**: 584–597.

36 Varela L, Horvath TL. Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis. EMBO Rep. 2012; **13**: 1079–1086.

37 Thon M, Hosoi T, Ozawa K. Possible integrative actions of leptin and insulin signaling in the hypothalamus targeting energy homeostasis. Front. Endocrinol. (Lausanne). 2016; **7**: 138.

38 Brown LM, Clegg DJ, Benoit SC, Woods SC. Intraventricular insulin and leptin reduce food intake and body weight in C57BL/6J mice. *Physiol Behav* 2006; **89**: 687–691.

39 Baskin DG, Wilcox BJ, Figlewicz DP, Dorsa DM. Insulin and insulin-like growth factors in the CNS. Trends Neurosci. 1988; **11**: 107–111.

40 Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature. 2006; **443**: 289–295.

41 Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC *et al.* Role of brain insulin receptor in control of body weight and reproduction. *Science* 2000; **289**: 2122–2125.

42 White MF. Insulin Signaling in Health and Disease. *Science* 2003; **302**: 1710–1711.

43 Coppari R, Ichinose M, Lee CE, Pullen AE, Kenny CD, McGovern RA *et al.* The hypothalamic arcuate nucleus: A key site for mediating leptin's effects on glucose homeostasis and locomotor activity. *Cell Metab* 2005; **1**: 63–72.

44 Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat. Genet. 1998; **18**: 214–215.

45 Sadaf Farooqi I, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. *Science (80- )* 2007; **317**: 1355.

46 Air EL, Benoit SC, Clegg DJ, Seeley RJ, Woods SC. Insulin and Leptin Combine Additively to Reduce Food Intake and Body Weight in Rats. *Endocrinology* 2002; **143**: 2449–2452.

47 Kleinridders A, Lauritzen HPMM, Ussar S, Christensen JH, Mori MA, Bross P *et al.* Leptin regulation of Hsp60 impacts hypothalamic insulin signaling. *J Clin Invest* 2013; **123**: 4667–4680.

48 Amitani M, Asakawa A, Amitani H, Inui A. The role of leptin in the control of insulin-glucose axis. *Front Neurosci* 2013. doi:10.3389/fnins.2013.00051.

49 Niswender KD, Schwartz MW. Insulin and leptin revisited: Adiposity signals with overlapping physiological and intracellular signaling capabilities. Front. Neuroendocrinol. 2003; **24**: 1–10.

50 Barr VA, Malide D, Zarnowski MJ, Taylor SI, Cushman SW. Insulin stimulates both leptin secretion and production by rat white adipose tissue. *Endocrinology* 1997; **138**: 4463–4472.

51 Tsubai T, Noda Y, Ito K, Nakao M, Seino Y, Oiso Y *et al.* Insulin elevates leptin secretion and mRNA levels via cyclic AMP in 3T3-L1 adipocytes deprived of glucose. *Heliyon* 2016; **2**: e00194.

52 Niswender KD, Magnuson MA. Obesity and the β cell: Lessons from leptin. *J Clin Invest* 2007; **117**: 2753–2756.

53 Chen PC, Kryukova YN, Shyng SL. Leptin regulates KATPchannel trafficking in pancreatic β-cells by a signaling mechanism involving AMP-activated protein kinase (AMPK) and camp-dependent protein kinase (PKA). *J Biol Chem* 2013; **288**: 34098–34109.

54 Ostlund RE, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab* 1996; **81**: 3909–3913.

55 Larsson H, Elmståhl S, Berglund G, Ahrén B. Evidence for Leptin Regulation of Food Intake in Humans. *J Clin Endocrinol Metab* 1998; **83**: 4382–4385.

56 Miller GD, Frost R, Olive J. Relation of plasma leptin concentrations to sex, body fat, dietary intake, and peak oxygen uptake in young adult women and men. *Nutrition* 2001; **17**: 105–111.

57 Yannakoulia M, Yiannakouris N, Blüher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab* 2003; **88**: 1730–1736.

58 Magni P, Liuzzi A, Ruscica M, Dozio E, Ferrario S, Bussi I *et al.* Free and bound plasma leptin in normal weight and obese men and women: Relationship with body composition, resting energy expenditure, insulin-sensitivity, lipid profile and macronutrient preference. *Clin Endocrinol (Oxf)* 2005; **62**: 189–196.

59 Murakami K, Sasaki S, Takahashi Y, Uenishi K, Yamasaki M, Hayabuchi H *et al.* Nutrient and food intake in relation to serum leptin concentration among young Japanese women. *Nutrition* 2007; **23**: 461–468.

60 Nakamura Y, Ueshima H, Okuda N, Murakami Y, Miura K, Kita Y *et al.* Serum leptin and total dietary energy intake: The INTERLIPID Study. *Eur J Nutr* 2013; **52**: 1641–1648.

61 Miller R, Tanofsky-Kraff M, Shomaker LB, Field SE, Hannallah L, Reina SA *et al.* Serum leptin and loss of control eating in children and adolescents. *Int J Obes* 2014; **38**: 397–403.

62 Tayyem RF, Hijjawi NS, Bawadi HA, Nimer NA, Agraib LM, Allehdan SS *et al.* Exploration of micro- and macronutrient consumption and leverage of interaction with adipokines among Jordanian adults. *Clin Pract* 2017; **15**: 410–423.

63 Hopkins M, Finlayson G, Duarte C, Gibbons C, Johnstone AM, Whybrow S *et al.* Biological and psychological mediators of the relationships between fat mass, fat-free mass and energy intake. *Int J Obes* 2019; **43**: 233–242.

64 Dore R, Levata L, Lehnert H, Schulz C. Nesfatin-1: Functions and physiology of a novel regulatory peptide. J. Endocrinol. 2017; **232**: R45–R65.

65 Goebel M, Stengel A, Wang L, Taché Y. Central nesfatin-1 reduces the nocturnal food intake in mice by reducing meal size and increasing inter-meal intervals. *Peptides* 2011; **32**: 36–43.

66 Pałasz A, Krzystanek M, Worthington J, Czajkowska B, Kostro K, Wiaderkiewicz R *et al.* Nesfatin-1, a unique regulatory neuropeptide of the brain. *Neuropeptides* 2012; **46**: 105–112.

67 Stengel A, Mori M, Taché Y. The role of nesfatin-1 in the regulation of food intake and body weight: Recent developments and future endeavors. Obes. Rev. 2013; **14**: 859–870.

68 Stengel A, Goebel M, Wang L, Rivier J, Kobelt P, Mönnikes H *et al.* Central Nesfatin-1 Reduces Dark-Phase Food Intake and Gastric Emptying in Rats: Differential Role of Corticotropin-Releasing Factor $_2$ Receptor. *Endocrinology* 2009; **150**: 4911–4919.

69 Yosten GLC, Samson WK. The anorexigenic and hypertensive effects of nesfatin-1 are reversed by pretreatment with an oxytocin receptor antagonist. *Am J Physiol Integr Comp Physiol* 2010; **298**: R1642–R1647.

70 Könczöl K, Pintér O, Ferenczi S, Varga J, Kovács K, Palkovits M *et al.* Nesfatin-1 exerts long-term effect on food intake and body temperature. *Int J Obes* 2012; **36**: 1514–1521.

71 Atsuchi K, Asakawa A, Ushikai M, Ataka K, Tsai M, Koyama K *et al.* Centrally administered nesfatin-1 inhibits feeding behaviour and gastroduodenal motility in mice. *Neuroreport* 2010; **21**: 1.

72 Yuan JH, Chen X, Dong J, Zhang D, Song K, Zhang Y *et al.* Nesfatin-1 in the lateral parabrachial nucleus inhibits food intake, modulates excitability of glucosensing neurons, and enhances UCP1 expression in brown adipose tissue. *Front Physiol* 2017; **8**: 235.

73 Pan W, Hsuchou H, Kastin AJ. Nesfatin-1 crosses the blood–brain barrier without saturation. *Peptides* 2007; **28**: 2223–2228.

74 Prinz P, Stengel A. Expression and regulation of peripheral NUCB2/nesfatin-1. Curr. Opin. Pharmacol. 2016; **31**: 25–30.

75 Stengel A, Hofmann T, Goebel-Stengel M, Lembke V, Ahnis A, Elbelt U *et al.* Ghrelin and NUCB2/nesfatin-1 are expressed in the same gastric cell and differentially correlated with body mass index in obese subjects. *Histochem Cell Biol* 2013; **139**: 909–918.

76 Stengel A, Goebel M, Yakubov I, Wang L, Witcher D, Coskun T *et al.* Identification and Characterization of Nesfatin-1 Immunoreactivity in Endocrine Cell Types of the Rat Gastric Oxyntic Mucosa. *Endocrinology* 2009; **150**: 232–238.

77 Ramanjaneya M, Chen J, Brown JE, Tripathi G, Hallschmid M, Patel S *et al.* Identification of nesfatin-1 in human and murine adipose tissue: A novel depot-specific adipokine with increased levels in obesity. *Endocrinology* 2010; **151**: 3169–3180.

78 Verdeguer F, Soustek MS, Hatting M, Blättler SM, McDonald D, Barrow JJ *et al.* Brown Adipose YY1 Deficiency Activates Expression of Secreted Proteins Linked to Energy Expenditure and Prevents Diet-Induced Obesity. *Mol Cell Biol* 2015; **36**: MCB.00722-15.

79 Shimizu H, Oh-I S, Hashimoto K, Nakata M, Yamamoto S, Yoshida N *et al.* Peripheral administration of nesfatin-1 reduces food intake in mice: The leptin-independent mechanism. *Endocrinology* 2009; **150**: 662–671.

80 Mortazavi S, Gonzalez R, Ceddia R, Unniappan S. Long-term infusion of nesfatin-1 causes a sustained regulation of whole-body energy homeostasis of male Fischer 344 rats. *Front cell Dev Biol* 2015; **3**: 22.

81 Tsuchiya T, Shimizu H, Yamada M, Osaki A, Oh-I S, Ariyama Y *et al.* Fasting concentrations of nesfatin-1 are negatively correlated with body mass index in non-obese males. *Clin Endocrinol (Oxf)* 2010; **73**: 484–490.

82 Psilopanagioti A, Nikou S, Papadaki H. Nucleobindin-2/nesfatin-1 in the human hypothalamus is reduced in obese subjects and colocalizes with oxytocin, vasopressin, melanin-concentrating hormone, and cocaine- and amphetamine-regulated transcript. *Neuroendocrinology* 2019; **108**(103):190-200.

83 Abaci A, Catli G, Anik A, Kume T, Bober E. The relation of serum nesfatin-1 level with metabolic and clinical parameters in obese and healthy children. *Pediatr Diabetes* 2013; **14**: 189–195.

84 Acar S, Çatli G, Küme T, Tuhan H, Gürsoy Çalan Ö, Demir K *et al.* Increased concentrations of serum nesfatin-1 levels in childhood with idiopathic chronic malnutrition. *Turkish J Med Sci* 2018; **48**: 378–385.

85 Hofmann T, Stengel A, Ahnis A, Bußе P, Elbelt U, Klapp BF. NUCB2/nesfatin-1 is associated with elevated scores of anxiety in female obese patients. *Psychoneuroendocrinology* 2013; **38**: 2502–2510.

86 Hofmann T, Elbelt U, Ahnis A, Rose M, Klapp BF, Stengel A. Sex-specific regulation of NUCB2/nesfatin-1: Differential implication in anxiety in obese men and women. *Psychoneuroendocrinology* 2015; **60**: 130–137.

87 Çelik F, Belviranli M, Okudan N. Circulating levels of leptin, nesfatin-1 and kisspeptin in postmenopausal obese women. *Arch Physiol Biochem* 2016; **122**: 195–199.

88 Tan BK, Hallschmid M, Kern W, Lehnert H, Randeva HS. Decreased cerebrospinal fluid/plasma ratio of the novel satiety molecule, nesfatin-1/NUCB-2, in obese humans: Evidence of nesfatin-1/NUCB-2 resistance and implications for obesity treatment. *J Clin Endocrinol Metab* 2011; **96**: E669–E673.

89 Kahraman FU, Vehapoğlu A, Özgen İT, Terzioğlu Ş, Cesur Y, Dündaröz R. Correlation of brain neuropeptide (Nesfatin-1 and orexin-a) concentrations with anthropometric and biochemical parameters in malnourished children. *JCRPE J Clin Res Pediatr Endocrinol* 2015; **7**: 197–202.

90 Anwar GM, Yamamah G, Ibrahim A, El-Lebedy D, Farid TM, Mahmoud R. Nesfatin-1 in childhood and adolescent obesity and its association with food intake, body composition and insulin resistance. *Regul Pept* 2014; **188**: 21–24.

91 Blanco AM, Bertucci JI, Delgado MJ, Valenciano AI, Unniappan S. Tissue-specific expression of ghrelinergic and NUCB2/nesfatin-1 systems in goldfish (Carassius auratus) is modulated by macronutrient composition of diets. *Comp Biochem Physiol -Part A Mol Integr Physiol* 2016; **195**: 1–9.

92 Chaolu H, Asakawa A, Ushikai M, Li YX, Cheng KC, Li JB *et al.* Effect of exercise and high-fat diet on plasma adiponectin and nesfatin levels in mice. *Exp Ther Med* 2011; **2**: 369–373.

93 Mori A, Momota Y, Miki Y, KIMURA T, ISHIOKA K, ODA H *et al.* Nutrients differentially regulate nucleobindin-2/nesfatin-1 in vitro in cultured stomach ghrelinoma (MGN3-1) cells and in vivo in male mice. *PLoS One* 2014; **9**: 411–417.

94 Butler AA, Zhang J, Price CA, Stevens JR, Graham JL, Stanhope KL *et al.* Low plasma adropin concentrations

increase risks of weight gain and metabolic dysregulation in response to a high-sugar diet in male nonhuman primates. *J Biol Chem* 2019; **294**(25):9706-9719.

95 Wong CM, Wang Y, Lee JTH, Huang Z, Wu D, Xu A *et al.* Adropin is a brain membrane-bound protein regulating physical activity via the NB-3/notch signaling pathway in mice. *J Biol Chem* 2014; **289**: 25976–25986.

96 Stein LM, Yosten GLC, Samson WK. Adropin acts in brain to inhibit water drinking: potential interaction with the orphan G protein-coupled receptor, GPR19. *Am J Physiol Integr Comp Physiol* 2016; **310**: R476–R480.

97 Thapa D, Xie B, Zhang M, Stoner MW, Manning JR, Huckestein BR *et al.* Adropin treatment restores cardiac glucose oxidation in pre-diabetic obese mice. *J Mol Cell Cardiol* 2019; **129**: 174–178.

98 Kumar KG, Zhang J, Gao S, Rossi J, McGuinness OP, Halem HH *et al.* Adropin Deficiency Is Associated With Increased Adiposity and Insulin Resistance. *Obesity* 2012; **20**: 1394–1402.

99 Akcılar R, Emel Koçak F, Şimşek H, Akcılar A, Bayat Z, Ece E *et al.* The effect of adropin on lipid and glucose metabolism in rats with hyperlipidemia. *Iran J Basic Med Sci* 2016; **19**: 245–51.

100 Gao S, McMillan RP, Jacas J, Zhu Q, Li X, Kumar GK *et al.* Regulation of substrate oxidation preferences in muscle by the peptide hormone adropin. *Diabetes* 2014; **63**: 3242–3252.

101 Gao S, McMillan RP, Zhu Q, Lopaschuk GD, Hulver MW, Butler AA. Therapeutic effects of adropin on glucose tolerance and substrate utilization in diet-induced obese mice with insulin resistance. *Mol Metab* 2015; **4**: 310–324.

102 Gao S, Ghoshal S, Zhang L, Stevens JR, McCommis KS, Finck BN *et al.* The peptide hormone adropin regulates signal transduction pathways controlling hepatic glucose metabolism in a mouse model of diet-induced obesity. *J Biol Chem* 2019; **294**: 13366–13377.

103 Butler AA, Tam CS, Stanhope KL, Wolfe BM, Ali MR, O'Keeffe M *et al.* Low Circulating Adropin Concentrations with Obesity and Aging Correlate with Risk Factors for Metabolic Disease and Increase after Gastric Bypass Surgery in Humans. *J Clin Endocrinol Metab* 2012; **97**: 3783–3791.

104 Sayin O, Tokgöz Y, Arslan N. Investigation of adropin and leptin levels in pediatric obesity-related nonalcoholic fatty liver disease. *J Pediatr Endocrinol Metab* 2014; **27**: 479–484.

105 Yosaee S, Khodadost M, Esteghamati A, Speakman JR, Shidfar F, Nazari MN *et al.* Metabolic Syndrome Patients Have Lower Levels of Adropin When Compared With Healthy Overweight/Obese and Lean Subjects. *Am J Mens Health* 2017; **11**: 426–434.

106 Zang H, Jiang F, Cheng X, Xu H, Hu X. Serum adropin levels are decreased in Chinese type 2 diabetic patients and negatively correlated with body mass index. *Endocr J* 2018; **65**: 685–691.

107 Choi H-N, Yim J-E. Plasma Adropin as a Potential Marker Predicting Obesity and Obesity-associated Cancer in Korean Patients With Type 2 Diabetes Mellitus. *J Cancer Prev* 2018; **23**: 191–196.

108 Ghoshal S, Stevens JR, Billon C, Girardet C, Sitaula S, Leon AS *et al.* Adropin: An endocrine link between the biological clock and cholesterol homeostasis. *Mol Metab* 2018; **8**: 51–64.

109 Lian W, Gu X, Qin Y, Zheng X. Elevated plasma levels of adropin in heart failure patients. *Intern Med* 2011; **50**: 1523–7.

110 Aydin S. Three new players in energy regulation: Preptin, adropin and irisin. *Peptides* 2014; **56**: 94–110.

111 Yildirim B, Celik O, Aydin S. Adropin: a key component and potential gatekeeper of metabolic disturbances in policystic ovarian syndrome. *Clin Exp Obstet Gynecol* 2014; **41**: 310–2.

112 Wu L, Fang J, Chen L, Zhao Z, Luo Y, Lin C *et al.* Low serum adropin is associated with coronary atherosclerosis in type 2 diabetic and non-diabetic patients. *Clin Chem Lab Med* 2014; **52**.

113 Kocaoglu C, Buyukinan M, Erdem SS, Ozel A. Are obesity and metabolic syndrome associated with plasma adropin levels in children? *J Pediatr Endocrinol Metab* 2015; **28**: 1293–7.

114 Altincik A, Sayin O. Evaluation of the relationship between serum adropin levels and blood pressure in obese children. *J Pediatr Endocrinol Metab* 2015; **28**: 1095–1100.

115 Zhang H, Jiang L, Yang Y-J, Ge R-K, Zhou M, Hu H *et al.* Aerobic exercise improves endothelial function and serum adropin levels in obese adolescents independent of body weight loss. *Sci Rep* 2017; **7**: 17717.

116 Chang JB, Chu NF, Lin FH, Hsu JT, Chen PY. Relationship between plasma adropin levels and body composition and lipid characteristics amongst young adolescents in Taiwan. *Obes Res Clin Pract* 2018; **12**: 101–107.

117 Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA *et al.* Adipose tissue dysfunction as

determinant of obesity-associated metabolic complications. *Int J Mol Sci* 2019; **20**: 2358.

118 Blüher M. Adipose tissue dysfunction contributes to obesity related metabolic diseases. *Best Pract Res Clin Endocrinol Metab* 2013; **27**: 163–177.

119 Zorena K, Jachimowicz-Duda O, Ślęzak D, Robakowska M, Mrugacz M. Adipokines and obesity. Potential link to metabolic disorders and chronic complications. *Int J Mol Sci* 2020; **21**(10):3570.

120 Khan S, Chan YT, Revelo XS, Winer DA. The Immune Landscape of Visceral Adipose Tissue During Obesity and Aging. Front. Endocrinol. (Lausanne). 2020; **11**: 267.

121 Rodríguez A, Ezquerro S, Méndez-Giménez L, Becerril S, Frühbeck G. Revisiting the adipocyte: a model for integration of cytokine signaling in the regulation of energy metabolism. *Am J Physiol Metab* 2015; **309**: E691–E714.

122 Cinti S. Pink Adipocytes. Trends Endocrinol. Metab. 2018; **29**: 651–666.

123 Luong Q, Lee KY. The Heterogeneity of White Adipose Tissue. In: *Adipose Tissue*. InTech, 2018 doi:10.5772/intechopen.76898.

124 Gómez-Hernández A, Beneit N, Díaz-Castroverde S, Escribano Ó. Differential Role of Adipose Tissues in Obesity and Related Metabolic and Vascular Complications. Int. J. Endocrinol. 2016; **2016:**1216783.

125 Sacks H, Symonds ME. Anatomical locations of human brown adipose tissue: Functional relevance and implications in obesity and type 2 diabetes. Diabetes. 2013; **62**: 1783–1790.

126 Kwok KHM, Lam KSL, Xu A. Heterogeneity of white adipose tissue: Molecular basis and clinical implications. Exp. Mol. Med. 2016; **48**: e215.

127 Servera M, López N, Serra F, Palou A. Expression of 'brown-in-white' adipocyte biomarkers shows gender differences and the influence of early dietary exposure. *Genes Nutr* 2014; **9**. doi:10.1007/s12263-013-0372-4.

128 Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH *et al.* Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 2012; **150**: 366–376.

129 Shinoda K, Luijten IHN, Hasegawa Y, Hong H, Sonne SB, Kim M *et al.* Genetic and functional characterization of clonally derived adult human brown adipocytes. *Nat Med* 2015; **21**: 389–394.

130 Lafontan M. Differences between subcutaneous and visceral adipose tissues. In: *Physiology and Physiopathology of Adipose Tissue*. Springer-Verlag France: Paris, 2013, pp 329–349.

131 Baglioni S, Cantini G, Poli G, Francalanci M, Squecco R, Franco A *et al.* Functional differences in visceral and subcutaneous fat pads originate from differences in the adipose stem cell. *PLoS One* 2012; 7(5):e36569.

132 Vidal H. Gene expression in visceral and subcutaneous adipose tissues. *Ann Med* 2001; **33**: 547–555.

133 Ibrahim MM. Subcutaneous and visceral adipose tissue: Structural and functional differences. Obes. Rev. 2010; **11**: 11–18.

134 Hwang I, Kim JB. Two faces of white adipose tissue with heterogeneous adipogenic progenitors. Diabetes Metab. J. 2019; **43**: 752–762.

135 Booth A, Magnuson A, Foster M. Detrimental and protective fat: Body fat distribution and its relation to metabolic disease. *Horm Mol Biol Clin Investig* 2014; **17**: 13–27.

136 Passaro A, Miselli MA, Sanz JM, Dalla Nora E, Morieri ML, Colonna R *et al.* Gene expression regional differences in human subcutaneous adipose tissue. *BMC Genomics* 2017; **18**: 1–11.

137 Lee M-J, Fried SK. Sex-dependent Depot Differences in Adipose Tissue Development and Function; Role of Sex Steroids. *J Obes Metab Syndr* 2017; **26**: 172–180.

138 Fitzgerald SJ, Janorkar AV, Barnes A, Maranon RO. A new approach to study the sex differences in adipose tissue. J. Biomed. Sci. 2018; **25**: 89.

139 Frank AP, De Souza Santos R, Palmer BF, Clegg DJ. Determinants of body fat distribution in humans may provide insight about obesity-related health risks. J. Lipid Res. 2019; **60**: 1710–1719.

140 Papadakis GE, Hans D, Rodriguez EG, Vollenweider P, Waeber G, Marques-Vidal P *et al.* Menopausal Hormone Therapy Is Associated with Reduced Total and Visceral Adiposity: The OsteoLaus Cohort. *J Clin Endocrinol Metab* 2018; **103**: 1948–1957.

141 Chang E, Varghese M, Singer K. Gender and Sex Differences in Adipose Tissue. Curr. Diab. Rep. 2018; **18**: 69.

142 Chao J, Rubinow KB, Kratz M, Amory JK, Matsumoto AM, Page ST. Short-term estrogen withdrawal increases

adiposity in healthy men. *J Clin Endocrinol Metab* 2016; **101**: 3724–3731.

143 Blouin K, Boivin A, Tchernof A. Androgens and body fat distribution. *J Steroid Biochem Mol Biol* 2008; **108**: 272–280.

144 Kim C, Harrall KK, Glueck DH, Shumer D, Dabelea D. Childhood adiposity and adolescent sex steroids in the Exploring Perinatal Outcomes among Children study. *Clin Endocrinol (Oxf)* 2019; **91**: 525–533.

145 Cosar E, Üçok K, Akgün L, Köken G, Sahin FK, Arioz DT *et al.* Body fat composition and distribution in women with polycystic ovary syndrome. *Gynecol Endocrinol* 2008; **24**: 428–432.

146 Dumesic DA, Akopians AL, Madrigal VK, Ramirez E, Margolis DJ, Sarma MK *et al.* Hyperandrogenism Accompanies Increased Intra-Abdominal Fat Storage in Normal Weight Polycystic Ovary Syndrome Women. *J Clin Endocrinol Metab* 2016; **101**: 4178–4188.

147 Li L, Feng Q, Ye M, He Y, Yao A, Shi K. Metabolic effect of obesity on polycystic ovary syndrome in adolescents: A meta-analysis. *J Obstet Gynaecol (Lahore)* 2017; **37**: 1036–1047.

148 Mondragón-Ceballos R, García Granados MD, Cerda-Molina AL, Chavira-Ramírez R, Hernández-López LE. Waist-to-hip ratio, but not body mass index, is associated with testosterone and estradiol concentrations in young women. *Int J Endocrinol* 2015; **2015**:654046.

149 Ghaben AL, Scherer PE. Adipogenesis and metabolic health. Nat. Rev. Mol. Cell Biol. 2019; **20**: 242–258.

150 Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O *et al.* Dynamics of fat cell turnover in humans. *Nature* 2008; **453**: 783–787.

151 Kim SM, Lun M, Wang M, Senyo SE, Guillermier C, Patwari P *et al.* Loss of white adipose hyperplastic potential is associated with enhanced susceptibility to insulin resistance. *Cell Metab* 2014; **20**: 1049–1058.

152 Vishvanath L, Gupta RK. Contribution of adipogenesis to healthy adipose tissue expansion in obesity. J. Clin. Invest. 2019; **129**: 4022–4031.

153 Heinonen S, Saarinen L, Naukkarinen J, Rodríguez A, Frühbeck G, Hakkarainen A *et al.* Adipocyte morphology and implications for metabolic derangements in acquired obesity. *Int J Obes* 2014; **38**: 1423–1431.

154 Hoffstedt J, Arner E, Wahrenberg H, Andersson DP, Qvisth V, Löfgren P *et al.* Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. *Diabetologia* 2010; **53**: 2496–2503.

155 Reddy P, Lent-Schochet D, Ramakrishnan N, McLaughlin M, Jialal I. Metabolic syndrome is an inflammatory disorder: A conspiracy between adipose tissue and phagocytes. Clin. Chim. Acta. 2019; **496**: 35–44.

156 Hocking SL, Stewart RL, Brandon AE, Suryana E, Stuart E, Baldwin EM *et al.* Subcutaneous fat transplantation alleviates diet-induced glucose intolerance and inflammation in mice. *Diabetologia* 2015; **58**: 1587–1600.

157 Foster MT, Shi H, Seeley RJ, Woods SC. Removal of intra-abdominal visceral adipose tissue improves glucose tolerance in rats: Role of hepatic triglyceride storage. *Physiol Behav* 2011; **104**: 845–854.

158 Singh M, Benencia F. Inflammatory processes in obesity: focus on endothelial dysfunction and the role of adipokines as inflammatory mediators. Int. Rev. Immunol. 2019; **38**: 157–171.

159 Chan P-C, Hsieh P-S. The Role of Adipocyte Hypertrophy and Hypoxia in the Development of Obesity-Associated Adipose Tissue Inflammation and Insulin Resistance. In: *Adiposity - Omics and Molecular Understanding*. InTech, 2017 doi:10.5772/65458.

160 Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab* 2007; **92**: 1023–1033.

161 Frühbeck G, Catalán V, Rodríguez A, Ramírez B, Becerril S, Salvador J *et al.* Involvement of the leptin-adiponectin axis in inflammation and oxidative stress in the metabolic syndrome. *Sci Rep* 2017; **7**: 1–8.

162 Frühbeck G, Catalán V, Rodríguez A, Gómez-Ambrosi J. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. Adipocyte. 2018; **7**: 57–62.

163 Frühbeck G, Catalán V, Rodríguez A, Ramírez B, Becerril S, Salvador J *et al.* Adiponectin-leptin ratio is a functional biomarker of adipose tissue inflammation. *Nutrients* 2019; **11**(2):454.

164 Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat. Rev. Immunol. 2011; **11**: 85–97.

165 Srikanthan K, Feyh A, Visweshwar H, Shapiro JI, Sodhi K. Systematic review of metabolic syndrome biomarkers: A panel for early detection, management, and risk stratification in the West Virginian population. Int. J. Med. Sci. 2016; **13**: 25–38.

166 Bussler S, Penke M, Flemming G, Elhassan YS, Kratzsch J, Sergeyev E *et al.* Novel Insights in the Metabolic Syndrome in Childhood and Adolescence. Horm. Res. Paediatr. 2017; **88**: 181–193.

167 Ghadge AA, Khaire AA. Leptin as a predictive marker for metabolic syndrome. Academic Press, 2019.

168 Reddy P, Lent-Schochet D, Ramakrishnan N, McLaughlin M, Jialal I. Metabolic syndrome is an inflammatory disorder: A conspiracy between adipose tissue and phagocytes. Clin. Chim. Acta. 2019; **496**: 35–44.

169 Choi J, Joseph L, Pilote L. Obesity and C-reactive protein in various populations: A systematic review and meta-analysis. Obes. Rev. 2013; **14**: 232–244.

170 Yuan G, Chen X, Ma Q, Qiao J, Li R, Li X *et al.* C-reactive protein inhibits adiponectin gene expression and secretion in 3T3-L1 adipocytes. *J Endocrinol* 2007; **194**: 275–281.

171 Yuan G, Jia J, Di L, Zhou L, Dong S, Ye J *et al.* Effects of C-reactive protein on adipokines genes expression in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 2012; **424**: 462–468.

172 Liu Y, Liu C, Jiang C, Wang S, Yang Q, Jiang D *et al.* C-reactive protein inhibits high-molecular-weight adiponectin expression in 3T3-L1 adipocytes via PI3K/Akt pathway. *Biochem Biophys Res Commun* 2016; **472**: 19–25.

173 Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003; **111**: 1805–1812.

174 Tillett WS, Francis T. Serological reactions in pneumonia with a nonprotein somatic fraction of pneumococcus. *J Exp Med* 1930; **52**: 561–71.

175 Berk BC, Weintraub WS, Alexander RW. Elevation of C-reactive protein in 'active' coronary artery disease. *Am J Cardiol* 1990; **65**: 168–172.

176 Ansar W, Ghosh S. *Biology of C reactive protein in health and disease*. 2016 doi:10.1007/978-81-322-2680-2.

177 Navarro P, de Dios O, Gavela-Pérez T, Jois A, Garcés C, Soriano-Guillén L. High-Sensitivity C-Reactive Protein and Leptin Levels Related to Body Mass Index Changes Throughout Childhood. *J Pediatr* 2016; **178**: 178–182.

178 Piening BD, Zhou W, Contrepois K, Röst H, Gu Urban GJ, Mishra T *et al.* Integrative Personal Omics Profiles during Periods of Weight Gain and Loss. *Cell Syst* 2018; **6**: 157-170.e8.

179 Li Q, Wang Q, Xu W, Ma Y, Wang Q, Eatman D *et al.* C-Reactive Protein Causes Adult-Onset Obesity Through Chronic Inflammatory Mechanism. *Front Cell Dev Biol* 2020; **8**: 18.

180 Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M *et al.* Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* 2003; **107**: 671–674.

181 Anty R, Bekri S, Luciani N, Saint-Paul MC, Dahman M, Iannelli A *et al.* The inflammatory C-reactive protein is increased in both liver and adipose tissue in severely obese patients independently from metabolic syndrome, type 2 diabetes, and NASH. *Am J Gastroenterol* 2006; **101**: 1824–1833.

182 Calabro P, Chang DW, Willerson JT, Yeh ET. Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: Linking obesity to vascular inflammation [1]. J. Am. Coll. Cardiol. 2005; **46**: 1112–1113.

183 Singh P, Hoffmann M, Wolk R, Shamsuzzaman ASM, Somers VK. Leptin induces C-reactive protein expression in vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 2007; **27**: 302–307.

184 De Rosa S, Cirillo P, Pacileo M, Di Palma V, Paglia A, Chiariello M. Leptin stimulated c-reactive protein production by human coronary artery endothelial cells. *J Vasc Res* 2009; **46**: 609–617.

185 Sun H, Zhang Y, Gao P, Li Q, Sun Y, Zhang J *et al.* Adiponectin reduces C-reactive protein expression and downregulates STAT3 phosphorylation induced by IL-6 in HepG2 cells. *Mol Cell Biochem* 2011; **347**: 183–189.

186 Chen K, Li F, Li J, Cai H, Strom S, Bisello A *et al.* Induction of leptin resistance through direct interaction of C-reactive protein with leptin. *Nat Med* 2006; **12**: 425–432.

187 Bianchi VE. The anti-inflammatory effects of testosterone. *J Endocr Soc* 2019; **3**: 91–107.

188 Traish A, Bolanos J, Nair S, Saad F, Morgentaler A. Do Androgens Modulate the Pathophysiological Pathways of Inflammation? Appraising the Contemporary Evidence. *J Clin Med* 2018; **7**: 549.

189 Joffe H V., Ridker PM, Manson JE, Cook NR, Buring JE, Rexrode KM. Sex hormone-binding globulin and serum testosterone are inversely associated with C-reactive protein levels in postmenopausal women at high risk for cardiovascular disease. *Ann Epidemiol* 2006; **16**: 105–112.

190 Störk S, Bots ML, Grobbee DE, van der Schouw YT. Endogenous sex hormones and C-reactive protein in healthy postmenopausal women. *J Intern Med* 2008; **264**: 245–253.

191 Zhang Y, Gao Y, Tan A, Yang X, Zhang H, Zhang S *et al.* Endogenous sex hormones and C-reactive protein in healthy chinese men. *Clin Endocrinol (Oxf)* 2013; **78**: 60–66.

192 Nakhai Pour HR, Grobbee DE, Muller M, Van Der Schouw YT. Association of endogenous sex hormone with C-reactive protein levels in middle-aged and elderly men. *Clin Endocrinol (Oxf)* 2007; **66**: 394–398.

193 Maggio M, Ceda GP, Lauretani F, Bandinelli S, Metter EJ, Artoni A *et al.* Estradiol and inflammatory markers in older men. *J Clin Endocrinol Metab* 2009; **94**: 518–522.

194 Kupelian V, Chiu GR, Araujo AB, Williams RE, Clark R V., McKinlay JB. Association of sex hormones and C-reactive protein levels in men. *Clin Endocrinol (Oxf)* 2010; **72**: 527–533.

195 Tsilidis KK, Rohrmann S, Mcglynn KA, Nyante SJ, Lopez DS, Bradwin G *et al.* Association between endogenous sex steroid hormones and inflammatory biomarkers in US men. *Andrology* 2013; **1**: 919–928.

196 Wander K, Brindle E, O'Connor KA. C-reactive protein across the menstrual cycle. *Am J Phys Anthropol* 2008; **136**: 138–146.

197 Blum CA, Müller B, Huber P, Kraenzlin M, Schindler C, De Geyter C *et al.* Low-grade inflammation and estimates of insulin resistance during the menstrual cycle in lean and overweight women. *J Clin Endocrinol Metab* 2005; **90**: 3230–3235.

198 Gaskins AJ, Wilchesky M, Mumford SL, Whitcomb BW, Browne RW, Wactawski-Wende J *et al.* Endogenous reproductive hormones and C-reactive protein across the menstrual cycle: The BioCycle Study. *Am J Epidemiol* 2012; **175**: 423–431.

199 Park J-M, Lee Y-J. Serum Estradiol Levels Are Inversely Associated With C-Reactive Protein in Premenopausal Women but Not Postmenopausal Women: a cross–sectional study. *J Int Med Res* 2020; **48**: 1–15.

200 Mogri M, Dhindsa S, Quattrin T, Ghanim H, Dandona P. Testosterone concentrations in young pubertal and post-pubertal obese males. *Clin Endocrinol (Oxf)* 2013; **78**: 593–599.

201 Wen X, Pekkala S, Wang R, Wiklund P, Feng G, Mei Cheng S *et al.* Does systemic low-grade inflammation associate with fat accumulation and distribution? A 7-year follow-up study with peripubertal girls. *J Clin Endocrinol Metab* 2014; **99**: 1411–1419.

202 Ağırbaşlı M, Tanrıkulu A, Azizy M. Free estradiol index levels associated with high sensitivity CRP levels in male children. *Turk J Pediatr* 2017; **59**: 49–55.

203 Sørensen K, Aksglaede L, Munch-Andersen T, Aachmann-Andersen NJ, Petersen JH, Hilsted L *et al.* Sex hormone-binding globulin levels predict insulin sensitivity, disposition index, and cardiovascular risk during puberty. *Diabetes Care* 2009; **32**: 909–914.

204 Pinkney J, Streeter A, Hosking J, Mohammod M, Jeffery A, Wilkin T. Adiposity, chronic inflammation, and the prepubertal decline of sex hormone binding globulin in children: evidence for associations with the timing of puberty (earlybird 58). *J Clin Endocrinol Metab* 2014; **99**: 3224–3232.

205 Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: A systematic literature review. *Nutr Rev* 2013; **71**: 511–527.

206 Lahoz C, Castillo E, Mostaza JM, de Dios O, Salinero-Fort MA, González-Alegre T *et al.* Relationship of the adherence to a mediterranean diet and its main components with CRP levels in the Spanish population. *Nutrients* 2018; **10**(3):379.

207 Navarro P, de Dios O, Jois A, Gavela-Pérez T, Gorgojo L, Martín-Moreno JM *et al.* Vegetable and fruit intakes are associated with hs-CRP levels in pre-pubertal girls. *Nutrients* 2017; **9**(3):224.

208 Louw JA, Werbeck A, Louw MEJ, Kotze TJVW, Cooper R, Labadarios D. Blood vitamin concentrations during the acute-phase response. *Crit Care Med* 1992; **20**: 934–941.

209 McMillan DC, Maguire D, Talwar D. Symposium 2: Nutrient interactions and their role in protection from chronic diseases: Relationship between nutritional status and the systemic inflammatory response: Micronutrients. *Proc Nutr Soc* 2019; **78**: 56–67.

210 Saboori S, Shab-Bidar S, Speakman JR, Yousefi Rad E, Djafarian K. Effect of Vitamin E supplementation on serum C-reactive protein level: A meta-analysis of randomized controlled trials. *Eur J Clin Nutr* 2015; **69**: 867–873.

211 Schwab S, Zierer A, Schneider A, Heier M, Koenig W, Kastenmüller G *et al.* Vitamin e supplementation is associated with lower levels of C-reactive protein only in higher dosages and combined with other antioxidants: The Cooperative Health Research in the Region of Augsburg (KORA) F4 study. *Br J Nutr* 2015; **113**: 1782–1791.

212 Kritchevsky SB, Bush AJ, Pahor M, Gross MD. Serum carotenoids and markers of inflammation in nonsmokers. *Am J Epidemiol* 2000; **152**: 1065–1071.

213 Erlinger TP, Guallar E, Miller ER, Stolzenberg-Solomon R, Appel LJ. Relationship between systemic markers of inflammation and serum beta-carotene levels. *Arch Intern Med* 2001; **161**: 1903–8.

214 Ford ES, Liu S, Mannino DM, Giles WH, Smith SJ. C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. *Eur J Clin Nutr* 2003; **57**: 1157–1163.

215 Il'Yasova D, Ivanova A, Morrow JD, Cesari M, Pahor M. Correlation between two markers of inflammation, serum C-reactive protein and interleukin 6, and indices of oxidative stress in patients with high risk of cardiovascular disease. *Biomarkers* 2008; **13**: 41–51.

216 Stephensen CB, Gildengorin G. Serum retinol , the acute phase response , and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey 1 – 3. *Am J Clin Nutr* 2000; **72**: 1170–1178.

217 Wood AD, Strachan AA, Thies F, Aucott LS, Reid DM, Hardcastle AC *et al.* Patterns of dietary intake and serum carotenoid and tocopherol status are associated with biomarkers of chronic low-grade systemic inflammation and cardiovascular risk. *Br J Nutr* 2014; **112**: 1341–1352.

218 Van Herpen-Broekmans W, Klöpping-Ketelaars I, Michiel B, Cornelis K, Hans P, Hendriks FJ *et al.* Serum carotenoids and vitamins in relation to markers of endothelial. *Eur J Epidemiol* 2004; **19**: 915–921.

219 Suarez EC, Schramm-Sapyta NL. Race differences in the relation of vitamins A, C, E, and β-carotene to metabolic and inflammatory biomarkers. *Nutr Res* 2014; **34**: 1–10.

220 Kongsbak K, Wahed M, Friis H, Thilsted S. Acute-phase protein levels, diarrhoea, Trichuris trichiura and maternal education are predictors of serum retinol: A cross-sectional study of children in a Dhaka slum, Bangladesh. *Br J Nutr* 2006; **96**: 725–734.

221 Maqsood M, Dancheck B, Gamble M V, Palafox NA, Ricks MO, Briand K *et al.* Vitamin A deficiency and inflammatory markers among preschool children in the Republic of the Marshall Islands. Nutr. J. 2004; **3**: 21.

222 García OP, Ronquillo D, Caamaño M del C, Martínez G, Camacho M, López V *et al.* Zinc, iron and vitamins A, C and E are associated with obesity, inflammation, lipid profile and insulin resistance in Mexican school-aged children. *Nutrients* 2013; **5**: 5012–5030.

223 Stephensen CB, Gildengorin G. Serum retinol , the acute phase response , and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey 1 – 3. *Am J Clin Nutr* 2000; **72**: 1170–1178.

224 Wang L, Gaziano JM, Norkus EP, Buring JE, Sesso HD. Associations of plasma carotenoids with risk factors and biomarkers related to cardiovascular disease in middle-aged and older women. *Am J Clin Nutr* 2008; **88**: 747–54.

225 Jois A, Navarro P, Ortega-Senovilla H, Gavela-Pérez T, Soriano-Guillén L, Garcés C. Relationship of high leptin levels with an adverse lipid and insulin profile in 6-8 year-old children in Spain. *Nutr Metab Cardiovasc Dis* 2015; **25**: 1111–1116.

226 Schoppen S, Riestra P, García-Anguita A, López-Simón L, Cano B, de Oya I *et al.* Leptin and adiponectin levels in pubertal children: relationship with anthropometric variables and body composition. *Clin Chem Lab Med* 2010; **48**: 707–11.

227 Navarro P, de Dios O, Gavela-Pérez T, Soriano-Guillen L, Garcés C. Relationship between polymorphisms in the CRP, LEP and LEPR genes and high sensitivity C-reactive protein levels in Spanish children. *Clin Chem Lab Med* 2017 Oct 26;55(11):1690-1695.

# 1. INFORMACIÓN DE LAS PUBLICACIONES INCLUIDAS EN LA TESIS DOCTORAL

Esta tesis doctoral se ha realizado por la modalidad de compendio de trabajos publicados en revistas científicas incluidas en el Journal Citation Reports (JCR). Las publicaciones se han citado según fecha de publicación o de propuesta en la revista en el caso de que el trabajo se encuentre en revisión.

1. **de Dios O**, Gavela-Pérez T, Aguado-Roncero P, Pérez-Tejerizo G, Ricote M, González N et al. C-reactive protein expression in adipose tissue of children with acute appendicitis. Pediatr Res 2018; 84: 564–567.

   INFORMACIÓN E INDICADORES DE CALIDAD:

   - Revista: Pediatric Research
   - Manuscrito recibido el 21 de noviembre de 2017; aceptado el 1 de junio de 2018.
   - Factor de impacto (JCR, 2019): 2,747.
   - Posición de la revista (categoría) (JCR, 2019): 18/125 (Pediatrics). Q1.

2. **de Dios O**, Navarro P, Ortega-Senovilla H, Herrero L, Gavela-Pérez T, Soriano-Guillen L et al. Plasma retinol levels and high-sensitivity C-reactive protein in prepubertal children. Nutrients 2018; 10(9), 1257.

   INFORMACIÓN E INDICADORES DE CALIDAD:

   - Revista: Nutrients
   - Manuscrito recibido el 21 de agosto de 2018; aceptado el 5 de septiembre de 2018.
   - Factor de impacto (JCR, 2019): 4,546.
   - Posición de la revista (categoría) (JCR, 2019): 17/89 (Nutrition & Dietetics). Q1.

3. **de Dios O**, Herrero L, Gavela-Pérez T, Soriano-Guillén L, Garcés C. Sex-specific association of plasma nesfatin-1 concentrations with obesity in children. Pediatr Obes 2019; 14(12):e12567.

   INFORMACIÓN E INDICADORES DE CALIDAD:

   - Revista: Pediatric Obesity.
   - Manuscrito recibido el 17 de febrero de 2019; aceptado el 5 de julio de 2019.
   - Factor de impacto (JCR, 2019): 3,429
   - Posición de la revista (categoría) (JCR, 2019): 13/128 (Pediatrics). Q1.

4. Herrero L, **de Dios O**, Gavela-Pérez T, Riestra P, Jois A, Soriano-Guillén L et al. Opposite Association of Adropin Concentrations with Obesity in Prepubertal Children Compared with Adolescents. Obesity 2020; 28: 1736–1741.

   INFORMACIÓN E INDICADORES DE CALIDAD:

   - Revista: Obesity.
   - Manuscrito recibido el 7 de abril de 2020; aceptado el 5 de mayo de 2020.
   - Factor de impacto (JCR, 2019): 3,742
   - Posición de la revista (categoría) (JCR, 2019): 49/143 (Endocrinology & Metabolism). Q2.

5. **de Dios O**, Herrero L, Vales-Villamarín C, Mahíllo-Fernández I, Soriano-Guillén L, Garcés C. Sex steroid hormones, leptin, and high-sensitivity C-reactive protein levels in adolescents. Andrology 2020. doi:10.1111/andr.12962.

   INFORMACIÓN E INDICADORES DE CALIDAD:

   - Revista: Andrology.
   - Manuscrito recibido el 6 de octubre de 2020; aceptado el 14 de diciembre de 2020.
   - Factor de impacto (JCR, 2019): 2,86.
   - Posición de la revista (categoría) (JCR, 2019): 1/8 (Andrology). Q1.

6. **de Dios O**, Vales-Villamarín C, Herrero L, Pérez-Segura P, Soriano-Guillén L, Garcés C. Analysis of leptin-adiponectin ratio and C-reactive protein as potential biomarkers of metabolic syndrome in adolescent. Clin Chem Lab Med 2021. doi:10.1515/cclm-2021-0366

   INFORMACIÓN E INDICADORES DE CALIDAD:

   - Revista: Clinical Chemistry and Laboratory Medicine
   - Manuscrito recibido el 9 de febrero de 2021; aceptado el 21 de abril de 2021.
   - Factor de impacto (JCR, 2019): 3,595.
   - Posición de la revista (categoría) (JCR, 2019): 5/29 (Medical Laboratory Technology). Q1.

7. **de Dios O**, Jois A, Herrero L, Vales-Villamarín C, Gavela-Pérez T,Gorjo L et al. Effect of leptin and insulin concentrations on dietary energy intake in children. Nutrition, Metabolism and Cardiovascular Diseases.

   INFORMACIÓN E INDICADORES DE CALIDAD:

   - Revista: Nutrition, Metabolism and Cardiovascular Diseases.
   - Manuscrito recibido el 1 de febrero de 2021; en revisión.
   - Factor de impacto (JCR, 2019): 3,7.
   - Posición de la revista (categoría) (JCR, 2019): 51/143 (Endocrinology & Metabolism). Q2.

# 2. OTRAS PUBLICACIONES NO INCLUIDAS EN LA TESIS DOCTORAL

## 2.1. PUBLICACIONES DIRECTAMENTE RELACIONADAS CON LA TESIS DOCTORAL

1. Pérez-Segura P, **de Dios O**, Herrero L, Vales-Villamarín C, Aragón-Gómez I, Gavela-Pérez T et al. Children with type 1 diabetes have elevated high-sensitivity C-reactive protein compared with a control group. BMJ Open Diabetes Res Care 2020; 8(1):e001424.
2. Lahoz C, Castillo E, Mostaza JM, **de Dios O,** Salinero-Fort MA, González-Alegre T, García-Iglesias F, Estirado E, Laguna F, Sanchez V, Sabín C, López S, Cornejo V, de Burgos C, Garcés C; Investigators of the SPREDIA-2 Group. Relationship of the Adherence to a Mediterranean Diet and Its Main Components with CRP Levels in the Spanish Population. Nutrients. 2018; 20;10(3):379.
3. Navarro P, **de Dios O**, Gavela-Pérez T, Soriano-Guillen L, Garcés C. Relationship between polymorphisms in the CRP, LEP and LEPR genes and high sensitivity C-reactive protein levels in Spanish children. Clin Chem Lab Med. 2017 Oct 26;55(11):1690-1695.
4. Navarro P, **de Dios O***, Jois A, Gavela-Pérez T, Gorgojo L, Martín-Moreno JM, Soriano-Guillen L, Garcés C. Vegetable and Fruit Intakes Are Associated with hs-CRP Levels in Pre-Pubertal Girls. Nutrients. 2017;9(3):224. ***Coautora.**
5. Navarro P, **de Dios O**, Gavela-Pérez T, Jois A, Garcés C, Soriano-Guillén L. High-Sensitivity C-Reactive Protein and Leptin Levels Related to Body Mass Index Changes Throughout Childhood. J Pediatr. 2016;178:178-182.

## 2.2. OTRAS PUBLICACIONES REALIZADAS DURANTE LA TESIS DOCTORAL

1. Mostaza JM, **de Dios O**, Lahoz C, Arribas M, Pérez Arroyo A, Salinero-Fort MA et al. Phenotype of haptoglobin and presence of subclinical vascular disease: Population study. Clin Investig Arterioscler. 2020;32(1):1-7.
2. Aganzo, M.; Montojo, M.-T.; López de Las Hazas, M.-C.; Martínez-Descals, A.; Ricote-Vila, M.; Sanz, R.; González-Peralta, I.; Martín-Hernández, R.; **de Dios, O**.; Garcés, C.; Galdón, A.; Lorenzo, Ó.; Tomás-Zapico, C.; Dávalos, A.; Vázquez, C.; González, N. Customized Dietary Intervention Avoids Unintentional Weight Loss and Modulates Circulating miRNAs Footprint in Huntington's Disease. Mol. Nutr. Food Res. 2018, e1800619.
3. Mostaza JM, Lahoz C, Salinero-Fort MA, **de Dios O,** Castillo E, González-Alegre T, García-Iglesias F, Estirado E, Laguna F, Sabín C, López S, Cornejo V, de Burgos C, Sanchez V, Garcés C; investigators of the SPREDIA-2 Group. R46L polymorphism in the PCSK9 gene: Relationship to lipid levels, subclinical vascular disease, and erectile dysfunction. J Clin Lipidol. 2018 ;12(4):1039-1046.e3.

# 3. Esquema resumen del trabajo de tesis doctoral